(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 June 2004 (03.06.2004)

PCT

(10) International Publication Number
WO 2004/046162 A2

(51) International Patent Classification[7]: C07J

(21) International Application Number:
PCT/US2003/036195

(22) International Filing Date:
14 November 2003 (14.11.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/426,456 14 November 2002 (14.11.2002) US
60/491,185 29 July 2003 (29.07.2003) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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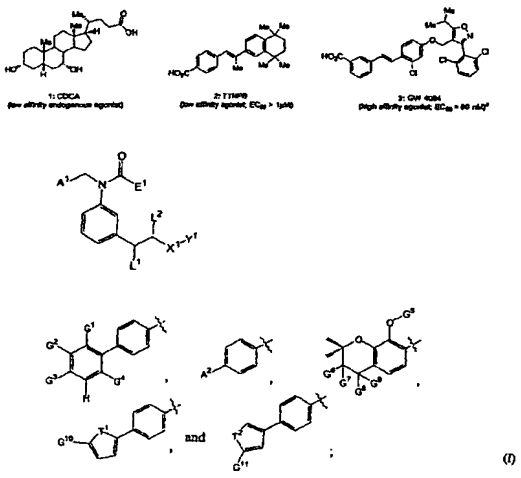
(54) Title: NON-STEROIDAL FXR AGONISTS



(I)

(57) Abstract: ABSTRACT Potent non-steroidal farnesoid X receptor (FXR) agonists are N-aryl-N-arylmethyl amido and ureido compounds having the chemical structure represented by the following formula (I): INSERT FORMULA wherein E1 is (C1-C8)alkyl, cyclohexyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, phenyl, or NH(C1-C8)alkyl; L1 and L2 are both H, or together form a pi-bond; X1 is C(O), or CH2; Y1 is H, NHZ1, NH(Z2)Z3, or OZ4; aryl moiety A1 is selected from the group of radicals consisting of: INSERT FORMULA A2 and G1 - G11 are as defined in the specification; and T1 and T2 are each independently O, S, NH, or N(C1-C8)alkyl. The FXR agonists are useful as therapeutic agents for the treatment of diseases linked to cholesterol, bile acids, and their metabolism and homeostasis.

WO 2004/046162 A2

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

NON-STEROIDAL FXR AGONISTS

Cross-reference to Related Applications

This application claims the benefit of U.S. Provisional Application Serial No. 60/426,456, filed on November 14, 2002, and U.S. Provisional Application Serial No. 60/491,185, filed on July 29, 2003, the disclosures of which are incorporated herein by reference.

Statement of Government Interest

This invention was made with government support under contract number CA-54418 from the National Institutes of Health. The U.S. government has certain rights in this invention.

Field of the Invention

The invention relates to agonists of farnesoid X receptor (FXR). More particularly, the invention relates to non-steroidal agonists of FXR, which are N-aryl-N-arylmethyl amido and ureido compounds. The agonists are useful for the regulation of cholesterol and related biological molecules.

Background

The efficient regulation of cholesterol biosynthesis, metabolism, acquisition, and transport is an essential component of lipid homeostasis. The farnesoid X receptor (FXR) is a transcriptional sensor for bile acids, the primary product of cholesterol metabolism. To date, only one class of high affinity, non-steroidal agonists for FXR has been reported, viz., the class exemplified by GW4064 (compound **3**, Figure 1), as reported by Maloney, P. R., et al. (*J. Med. Chem.* **2000**, *43*, 2971–2974). Potent steroid-derived agonists of FXR have been reported by Pellicciari, R., et al. (*J. Med. Chem.* **2002**, *45*, 3569–3572).

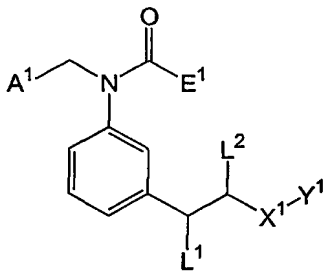
Potent, selective, non-steroidal small molecule FXR agonists are powerful tools for exploring the biological function of the farnesoid X receptor and would have many other useful applications (Willson, T. M., et al., *Med. Res. Rev.* **2001**, *21*, 513–522). For example, such compounds facilitate

the analysis of FXR physiology *in vivo*, and in conjunction with DNA arraying technology facilitate discovery of new gene products under the control of FXR. FXR modulators also are useful in the treatment of cholestasis and other disease states associated with aberrant levels, flow, and release of bile acids. The crystal structure of FXR has not yet been reported. There is an unfulfilled need for a thorough structure/activity relationship (SAR) study of materials that modulate FXR activity, and for novel and potent ligands and therapeutic agents targeted to FXR. The non-steroidal FXR agonists of the present invention fulfill these needs.

Summary of the Invention

The non-steroidal FXR agonists of the present invention are N-aryl-N-arylmethyl amido and ureido compounds having the chemical structure represented by the following formula (I):

(I)



wherein

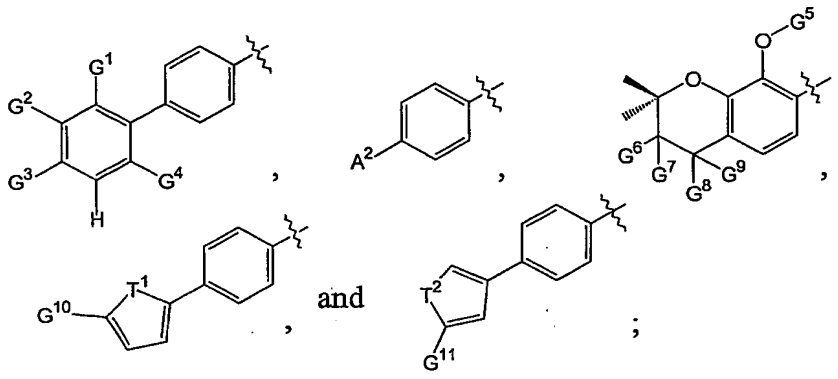
electrophile-derived moiety E^1 is $(C_1\text{-}C_8)$alkyl, cyclohexyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, phenyl, or $NH(C_1\text{-}C_8)$alkyl;

L^1 and L^2 are both H, or together form a pi-bond;

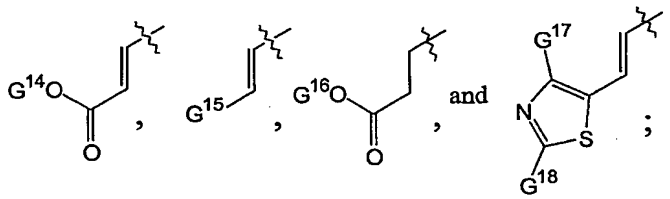
X^1 is C(O), or CH_2;

Y^1 is H, NHZ^1, $NH(Z^2)Z^3$, or OZ^4;

aryl moiety A^1 is selected from the group of radicals consisting of:



A^2 is a radical selected from the group consisting of:



substituent group G^1 is H or OCH_3;

G^2 and G^3 are each independently H, $(C_1\text{-}C_8)$alkyl, F, Cl, Br, I, OH, $O(C_1\text{-}C_8)$alkyl, SH, $S(C_1\text{-}C_8)$alkyl, C(O)H, $C(O)(C_1\text{-}C_8)$alkyl, $N((C_1\text{-}C_8)alkyl)_2$, CO_2CH_3, or together form a 5 or 6-member carbocyclic ring or OCH_2O;

G^4 is H or OCH_3;

G^5 is $(C_1\text{-}C_4)$alkyl or $C(O)(C_1\text{-}C_8)$alkyl;

G^6 is H, or together with G^8 forms a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

G^7 is H, CH_3, or OZ^5, with the proviso that G^7 is H or CH_3 when G^6 and G^8 together form a pi-bond, an epoxide, a cyclopropyl ring, a

dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

when G^6 is H, G^8 is H, or together with G^9 forms a moiety selected from the group consisting of =NOZ^6 and =O;

G^9 is H, OH, OZ^7, CN, C(O)O(C_1-C_8)alkyl, SPh, S(C_1-C_8)alkyl, NHZ^8, $NH(Z^9)Z^{10}$, or together with G^{10} forms a moiety selected from the group consisting of =NOZ^6 and =O;

G^{10} and G^{11} are each independently H, (C_1-C_8)alkyl, SCH_3, C(O)(C_1-C_8)alkyl, or C(O)O(C_1-C_8)alkyl; and

G^{12} and G^{13} are each independently H or F;

G^{14} and G^{16} are each independently (C_1-C_8)alkyl, phenyl, or benzyl;

G^{15} is phenyl, (C_1-C_8)alkylphenyl; hydroxyphenyl, (C_1-C_8)alkoxyphenyl, chlorophenyl, bromophenyl, or fluorophenyl; and

G^{17} and G^{18} are each independently H, (C_1-C_8)alkyl, SCH_3, C(O)(C_1-C_8)alkyl, or C(O)O(C_1-C_8)alkyl;

T^1 and T^2 are each independently O, S, NH, or N(C_1-C_8)alkyl;

Z^1 is H, phenyl, (C_1-C_8)alkyl, benzyl, C(O)Ph, C(O)(C_1-C_8)alkyl, C(O)OCH_2Ph, or C(O)NH(C_1-C_8)alkyl;

Z^2 and Z^3 are each independently (C_1-C_8)alkyl or together form a (C_1-C_8)cycloalkyl ring;

Z^4, Z^5, Z^6, and $Z^7$are each independently H or an oxygen protecting group;

Z^8 is H, phenyl, (C_1-C_8)alkyl, benzyl, C(O)Ph, C(O)(C_1-C_8)alkyl, C(O)OCH_2Ph, or C(O)NH(C_1-C_8)alkyl;

Z^9 and Z^{10} are each independently (C_1-C_8)alkyl, or together form a (C_5-C_8)cyclic amine ring.

Typical examples of the FXR agonists of the present invention are illustrated in Figure 2. Several of these compounds are among the most potent FXR activators reported to date. Preferred examples of the FXR agonists of the present invention are fexaramate (**105**), fexarene (**121**),

fexaramine (**259**), fexarine (**244**), and fexarchloramide (**149**) as shown in Figure 2. The FXR agonists of the present invention can be employed as therapeutic agents for the treatment of diseases linked to cholesterol, bile acids, and their metabolism and homeostasis, and are useful as tools for elucidation of FXR biological function.

Brief Description of Drawings

Figure 1 illustrates structures of some natural and synthetic agonists of FXR (farnesoid X receptor) and their activity in a cell based assay.

Figure 2 illustrates structures of 9 non-steroidal FXR agonists of the present invention and their EC_{50} values obtained from a cell-based assay.

Figure 3(a) illustrates structures of selected hits from a high throughput screen for FXR agonism of a 10,000-member benzopyran-based natural product-like library (EC50 = 5-10 mM). Figure 3(b) shows the structures of selected low affinity FXR agonists from a follow-up solid phase benzopyran library (EC50 = 5-10 mM). The boxed compounds represent the most potent FXR agonists in this group.

Figure 4 schematically illustrates the solid-phase synthesis of a focused library of benzopyran-containing small molecules as potential FXR agonists. Figure 4(a) shows the solid-phase protocol. Figure 4(b) shows O-prenylated phenols employed as scaffolds. Figure 4(c) shows the structures of the electrophiles utilized in the acylation step in the transformation of S-2 to S-3 shown in Figure 4(a). Figure 4(d) shows the structures of the amines employed in the reductive amination step in the transformation of S-2 to S-3 shown in Figure 4(a). The reagents and conditions for these reactions are well known in the art and have been reported in Nicolaou, K. C.; et al. *J. Am. Chem. Soc.* **2000**, *122*, 9939-9953, the relevant disclosures of which are incorporated herein by reference.

Figure 5 illustrates selected regions of interest for SAR evaluation of lead compound **26**. Region I: Right-hand aromatic system; Region II: Acyl group region; Region III: Left-hand benzopyran ring system.

Figure 6 illustrates the structural variants of Region I that were examined in a SAR study. See Figures 7, 8, 9 and 10 for a schematic illustration of the synthesis of these compounds. In compound **46** the benzopyran double bond was hydrogenated. The boxed compounds represent the most potent FXR agonists within this group of compounds.

Figure 7 schematically illustrates the representative procedure for the preparation of Region I-modified compounds: synthesis of methyl acrylate **29**. Reagents and conditions: (a) (Glass, C. K.; et al. *Curr. Opin. Cell Biol.* **1997**, *9*, 222-232; (b) 1.5 equivalents of 2-methyl-3-butyn-2-ol, 1.5 of DBU, 1.7 equivalents trifluoroacetic anhydride, 0.1 equivalents of $CuCl_2$, CH_3CN, 0 → 25 °C,12 h, 75%; (c) N,N-diethylaniline, 190 °C, 0.5 h, 90%; (d) 1.5 equivalents of 3-bromoaniline, THF, 70 °C, 4h; then 2.0 equivalents of $NaCNBH_3$, 10% MeOH, 70 °C, 4h, 83%; (e) 1.3 equivalents of cyclopropanecarbonyl chloride, 1.3 equivalents of Et_3N, 0.1 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 12 h, 90%; (f) 4.0 equivalents of methyl acrylate, 0.2 equivalents of $Pd_2(dba)_3$, 0.5 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 80%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, P(*o*-tol)$_3$ = tris-(2-methylphenyl)phosphine, 4-DMAP = 4-dimethylaminopyridine, $Pd_2(dba)_3$ = tris(dibenzylidineacetone)dipalladium(0).

Figure 8 schematically illustrates the solution phase synthesis of ester and acid containing compounds (SAR region I). Reagents and conditions: (a) (Glass, C. K.; et al. *Curr. Opin. Cell Biol.* **1997**, *9*, 222-232; (b) 1.5 equivalents of 2-methyl-3-butyn-2-ol, 1.5 equivalents of DBU, 1.7 equivalents of trifluoroacetic anhydride, 0.1 equivalents of $CuCl_2$, CH_3CN, 0 → 25 °C,12 h, 75%; (c) N,N-diethylaniline, 190 °C, 0.5 h, 90%; (d) 1.5 equivalents of methyl 4-aminobenzoate, THF, 70 °C,4 h; then 2.0 equivalents of $NaCNBH_3$, 10% MeOH, 70 °C, 4 h, 82%; (e) 1.3 equivalents of cyclopropanecarbonyl chloride, 1.3 equivalents of Et_3N, 0.1 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 12 h, 85-95%; (f) 1.5 equivalents of ethyl 3-aminobenzoate, THF , 70 °C, 4 h; then 2.0 equivalents of $NaCNBH_3$, 10%

MeOH, 70 °C, 4h, 77%; (g) 1.5 equivalents of methyl 4-(aminomethyl)benzoate, THF, 70 °C, 4h; then 2.0 equivalents of $NaCNBH_3$,10% MeOH, 70 °C, 4h, 80% ; (h) 4.0 equivalents of LiOH, THF:H_2O (10:1), 25 °C, 12 h, 75-98%; (i) 1.5 equivalents of 4-bromoaniline, THF, 70 °C, 4h; then 2.0 equivalents of $NaCNBH_3$, 10% MeOH, 70 °C, 4h, 78%; (j) 4.0 equivalents of methyl acrylate, 0.2 equivalents of $Pd_2(dba)_3$, 0.5 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 71-80%; (k) 1.5 equivalents of 3-bromoaniline, THF, 70 °C, 4h; then 2.0 equivalents of $NaCNBH_3$, 10% MeOH, 70 °C, 4h, 83%; (l) 1.3 equivalents of cyclohexanecarbonyl chloride, 1.3 equivalents of Et_3N, 0.1 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 12 h, 95%.

Figure 9 schematically illustrates the solution phase synthesis of various ester and vinyl cyanide containing compounds via palladium catalyzed reaction manifolds (SAR region I). Reagents and conditions: (a) 2.0 equivalents of penta-2,4-dienoic acid methyl ester, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 70%; (b) 5.0 equivalents of 3-(methoxycarbonylphenyl)boronic acid, toluene:MeOH:1M Na_2CO_3 (10:3:1), 90 °C, 24 h, 75%; (c) 5.0 equivalents of 4-(methoxycarbonylphenyl)boronic acid, toluene:MeOH:1M Na_2CO_3 (10:3:1), 90 °C, 24 h, 78%; (d) 2.0 equivalents of 3-vinylbenzaldehyde, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 85%; (e) 1.5 equivalents of $NaClO_2$, 4.0 equivalents of NaH_2PO_4, 10.0 equivalents of 2-methyl-2-butene, THF:*t*-BuOH:H2O (3:1:1), 25 °C, 3 h, 98% ; (f) 10 equivalents of CH_2N_2, Et_2O, 0 °C, 1 h, 100%; (g) 2.0 equivalents of acrylonitrile, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24h, 55%.

Figure 10 schematically illustrates the solution phase synthesis of ester modifications (SAR region I). Reagents and conditions: (a) 0.5 equivalents of *n*-Bu_2Sn=O, EtOH or *i*-PrOH, 25 °C, 48 h, 50% and 34%, respectively; (b) 1.2 equivalents of diisobutylaluminum hydride, toluene, -78

°C, 0.5 h, 52%; (c) 2.0 equivalents of NaH, 3.0 equivalents of MeI, 0 °C, 1 h, 95%; (d) 1.2 equivalents of MeOC(O)Cl, 2.0 equivalents of Et_3N, 0.1 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 24 h, 88%; (e) 1.2 equivalents of MeC(O)Cl, 2.0 equivalents of Et_3N, 0.1 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 24 h, 90%; (f) 4.0 equivalents of LiOH, THF:H_2O (10:1), 25 °C, 12h, 90%; (g) 1.2 equivalents of EtOC(O)Cl, 1.5 equivalents of Et_3N, CH_2Cl_2, 25 °C,1 h, then 3.0 equivalents of amine, CH_2Cl_2, 25 °C,12 h, 85-95%; (h) 10.0 equivalents of CH_2N_2, 0.2 equivalents of $Pd(OAc)_2$, Et_2O, 25 °C, 12 h, 95%.

Figure 11 depicts the structures of compounds in which the acyl group of region II was varied. See Figure 12 for a schematic representation of the synthesis of these compounds. Boxed compounds are the most active FXR agonists within this group.

Figure 12 schematically illustrates the solution phase synthesis of ester modifications (SAR region II). Reagents and conditions: (a) 1.0 equivalents of **60**, 2.0 equivalents of **130**, THF, 70 °C, 4 h, then 2.0 equivalents of $NaCNBH_3$, 10% MeOH, 70 °C, 4 h, 70%; (b) 1.5 equivalents of methyl acrylate, 0.2 equivalents of $Pd_2(dba)_3$, 0.5 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 12 h, 65%; (c) 5.0 equivalents of $NaHCO_3$, 5.0 equivalents of alkyl halide, EtOH, 80 °C, 24 h, 70-85%; (d) 5.0 equivalents of acid chloride, 5.0 equivalents of Et_3N, 0.2 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 24 h, 55-100%; (e) 5.0 equivalents of isocyanate, 5.0 equivalents of Et_3N, CH_2Cl_2, 25 °C, 24 h, 75-85%; (f) 5.0 equivalents of thioacid chloride or thioisocyanate, 5.0 equivalents of Et_3N, CH_2Cl_2, 25 °C, 24h, 50-70%.

Figure 13 depicts the structures of compounds in which the acyl group of region II was varied. See Figures 14, 15, and 16 for a schematic representation of the synthesis of these compounds. Boxed compounds are the most active FXR agonists in this group.

Figure 14 schematically illustrates the solution phase synthesis of benzopyran olefin modifications (SAR region III). Reagents and conditions: (a) 2.0 equivalents of benzoyl chloride, 2.0 equivalents of Et_3N, 0.2 equivalents

of 4-DMAP, CH_2Cl_2, 25 °C, 24 h, 95%; (b) 10 equivalents of DMDO, acetone, 0 °C, 1 h, 100%; (c) 5.0 equivalents of PhSH, Amberlyst-15 (cat.), CH_2Cl_2 , 25 °C, 24 h, 95%; (d) 2.0 equivalents of acetic anhydride, 2.0 equivalents of Et_3N, 0.2 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 24 h, 90%; (e) 2.0 equivalents of methyl acrylate, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 70-84%; (f) 5.0 equivalents of piperidine, CH_2Cl_2, 25 °C, 48h, 65%; (g) 5.0 equivalents of H_2O, Amberlyst-15 (cat.), THF , 25 °C, 24 h, 95%; (h) 2.0 equivalents of Et_2AlCN, CH_2Cl_2, 0 °C, 1 h, 83%; (i) 40% KOH:MeOH (1:2), 25 °C,24 h, 90%. DMDO = dimethyldioxirane.

Figure 15 schematically illustrates the solution phase synthesis of benzopyran olefin modifications (SAR region III). Reagents and conditions: (a) 0.02 equivalents of OsO_4, 2.0 equivalents of NMO, acetone:H_2O (10:1), 25 °C, 24 h, 85%; (b) 5.0 equivalents of acetic anhydride, 10.0 equivalents of Et_3N, 0.2 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 24 h, 90%; (c) 2.0 equivalents of methyl acrylate, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 65-80%; (d) 10% Pd/C, EtOAc, 25 °C, 0.5 h, 100%; (e) $CHCl_3$: 2.0 N NaOH (7:1), adogen 464 (cat.) 25 °C, 6 h, 85%. NMO = 4-methylmorpholine N-oxide.

Figure 16 schematically illustrates the synthesis of compound **102** (Modifications of region III SAR). Reagents and conditions: (a) $CHCl_3$: 2.0 N NaOH (7:1), adogen 464 (cat.) 25 °C, 6 h, 85%; (b) 2.0 equivalents of methyl acrylate, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, 5.0 equivalents of Et_3N, DMF, 90 °C, 24 h, 75%. DMF = N,N-dimethylformamide.

Figure 17 depicts the structures utilized for examining the region III benzopyran replacement SAR study. See Figures 21, 18, 19, and 25 for a schematic representation of the synthesis of these compounds.

Figure 18 schematically illustrates the solution phase synthesis of region III analogs in which the benzopyran group has been replaced.

Reagents and conditions: (a) 1.1 equivalents of $C_6H_{11}COCl$, 1.3 equivalents of Et_3N, 0.05 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 3 h, 95%; (b) 4.0 equivalents of methyl acrylate, 5.0 equivalents of Et_3N, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 80% ; (c) 1.1 equivalents of NaH, THF, 0 °C, 30 min; then 1.3 equivalents of benzyl bromides, THF, 0 °C, 2 h, 60 - 90%. R-X = methyl iodide, benzyl bromide, 2-bromobenzyl bromide, 3-bromobenzyl bromide, 4-bromobenzyl bromide, 4-tert-butylbenzyl bromide, 3-methoxybenzyl bromide, 3,5-dimethoxybenzyl bromide, 3-(trifluromethyl)benzyl bromide, 2-napthyl bromide.

Figure 19 schematically illustrates the solution phase synthesis of region III derivatives. Reagents and conditions: (a) 4.0 equivalents of *tert*-butyl acrylate, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 80%; (b) 20% TFA in CH_2Cl_2, 25 °C, 1 h, 95%.

Figure 20 shows the structures of compounds from the SAR studies. Figure 20(a) illustrates highlights of the region I SAR. Figure 20(b) illustrates highlights of the region II SAR for bis-cinnamate compounds. Figure 20(c) illustrates effects of benzopyran substitution. Figure 20(d) illustrates highlights of the region III SAR, including bis-cinnamate, styryl and biaryl compounds. The EC_{50} values represent the mean of at least four measurements. RE = relative efficacy of the indicated compound at 1 mM to 100 mM CDCA.

Figure 21 schematically illustrates the preparation of the bis-cinnamate compound **105**. Reagents and conditions: (a) 1.1 equivalents of $C_6H_{11}COCl$, 1.3 equivalents of Et_3N, 0.05 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 3 h, 95%; (b) 4.0 equivalents of methyl acrylate, 5.0 equivalents of Et_3N, 0.2 equivalents of $Pd_2(dba)_3$, 0.6 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 80%; (c) 1.1 equivalents of NaH, THF, 0 °C, 30 min; then 1.3 equivalents of 4-bromobenzylbromide, THF, 0 °C, 2 h, 90%; (d) 4.0 equivalents of acrylate, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of

P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 75%.

Figure 22 schematically illustrates the synthesis of analogs with region III modifications and cinnamate substitutions. Reagents and conditions: (a) 4.0 equivalents of styrene, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 65 - 80% ; (b) 2.5 equivalents of boronic acid, 0.2 equivalents of $Pd(PPh_3)_4$, toulene:MeOH:1 M Na_2CO_3 (10:3:1), 80 °C, 12 h, 60 - 80%.

Figure 23 schematically illustrates the synthesis of analogs having region I/region III cinnamate modifications. Reagents and conditions: (a) 4.0 equivalents of *tert*-butyl acrylate, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 85%; (b) 1.5 equivalents of 3-bromoaniline, 0.05 equivalents of AcOH, MeOH, 25 °C, 30 min; then 1.7 equivalents of $NaCNBH_3$, 1 h, 90%; (c) 1.1 equivalents of $C_6H_{11}COCl$, 1.3 equivalents of Et_3N, 0.05 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 3 h, 90%; (d) 4.0 equivalents of acrylate, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 60 - 85%; (e) 4.0 equivalents of alkene, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 35 - 80%; (f) 0.05 equivalents of Pd/C, H_2 (1 atm), EtOAc, 25 °C, 30 min, 100 %.

Figure 24 schematically illustrates the synthesis of acyl group analogs of the bis-cinnamate compounds. Reagents and conditions: (a) 1.0 equivalents of **S-24**, 1.0 equivalents of **S-27**, 0.05 equivalents of AcOH, MeOH, 25 °C, 30 min; then 1.2 equivalents of $NaCNBH_3$, 25 C, 1 h, 85%; (b) 2.0 equivalents of acid chloride, 3.0 equivalents of Et_3N, 0.05 equivalents of 4-DMAP, CH_2Cl_2, 25 C, 1 h, 80 - 95%; (c) 2.0 equivalents of isocyanate, 3.0 equivalents of Et_3N, 0.05 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 1 h, 60 - 80%.

Figure 25 schematically illustrates the synthesis of region III cinnamate modifications. Reagents and conditions: (a) 4.0 equivalents of acrylate, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15

equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 50 - 80% ; (b) 20% TFA in CH_2Cl_2, 1 h, 25 C, 95%; (c) 1.2 equivalents of DCC, 10.0 equivalents of *i*-PrOH, 0.2 equivalents of 4-DMAP, DMF, 25 °C, 12 h, 60%; (d) 1.2 equivalents of DCC, 10.0 equivalents of BnOH, 0.2 equivalents of 4-DMAP, DMF, 25 °C, 12 h, 60%; (e) 4.0 equivalents of alkene, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 12 h, 35 - 75%; (f) 0.05 equivalents of Pd/C, H_2 (1 atm), EtOAc, 25 °C, 30 min, 100 %. DCC = 1,3-dicyclohexylcarbodiimide.

Figure 26 schematically illustrates the synthesis of region III ring analogs. Reagents and conditions: (a) 1.0 equivalents of SEMCl, 1.2 equivalents of Et_3N, CH_2Cl_2, 25 °C, 12 h, 75%; (b) 1.05 equivalents of Tf_2O, 1.2 equivalents of Et_3N, CH_2Cl_2, -78 °C, 1 h, 95%; (c) 4.0 equivalents of tert-butyl acrylate, 5.0 equivalents of Et_3N, 0.05 equivalents of $Pd_2(dba)_3$, 0.15 equivalents of P(*o*-tol)$_3$, 90 °C, 12 h, 76%; (d) 1.2 equivalents of **S-27**, 0.05 equivalents of AcOH, MeOH, 25 °C, 1 h; then 1.5 equivalents of $NaCNBH_3$, 2 h, 80%; (e) 1.2 equivalents of $C_6H_{11}COCl$, 1.5 equivalents of Et_3N, 0.05 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 4 h, 90%; (f) 7.0 equivalents of TBAF, THF:HMPA (9:1), 55 °C, 12 h, 65%; (g) 3.0 equivalents of MeI, 5.0 equivalents of K_2CO_3, DMF, 80 °C, 12 h, 90%; (h) 3.0 equivalents of BnBr, 5.0 equivalents of K_2CO_3, DMF, 80 °C, 12 h, 65%; (i) 3.0 equivalents of $BrCH_2COOEt$, 5.0 equivalents of K_2CO_3, DMF, 80 °C, 12 h, 85%; (j) 3.0 equivalents of AcCl, BzCl or MsCl, 5.0 equivalents of Et_3N, CH_2Cl_2, 25 °C, 2 h, 70-90%. HMPA = hexamethylphosphoramide, Tf_2O = trofluoroacetic anhydride, TBAF = tetrabutylammonium fluoride, SEMCl = 2-(trimethylsilyl)ethoxymethyl chloride.

Figure 27 schematically illustrates the solid phase synthesis of focused libraries of biaryl and stilbene cinnamates. Reagents and conditions: (a) 2.0 equivalents of **168**, 1.0 equivalents of Merrifield Resin (0.91 mmol/g), 2.0 equivalents of Cs_2CO_3, 0.5 equivalents of TBAI, DMF, 55 °C, 24 h; (b) 20% TFA in CH_2Cl_2, 25 °C, 1 h; (c) 10.0 equivalents of 4-bromobenzaldehyde,

0.05 equivalents of AcOH, THF:MeOH (2:1), 25 °C, 1 h; then, 8.0 equivalents of $NaCNBH_3$, THF:MeOH (2:1), 25 °C, 2 h; (d) for $R_1C(O)Cl$: 30.0 equivalents of *i*-PrC(O)Cl or $C_6H_{11}C(O)Cl$, 40.0 equivalents of Et_3N, 1.0 equivalents of 4-DMAP, CH_2Cl_2, 25 °C, 12 h; for R_1NCO, 30.0 equivalents of i-PrNCO, 40.0 equivalents of Et_3N, 1.0 equivalents of 4-DMAP, DMF, 65 °C, 60 h; (e) 8.0 equivalents of styrene, 10.0 equivalents of Et_3N, 0.5 equivalents of $Pd_2(dba)_3$, 1.5 equivalents of P(*o*-tol)$_3$, DMF, 90 °C, 48 h; (f) 5.0 equivalents of boronic acid, 3.0 equiv Cs_2CO_3, 0.5 equivalents of $Pd(PPh_3)_4$, DMF, 90 °C, 24 h; (g) 10.0 equivalents of NaOMe, Et_2O:MeOH (10:1), 25 °C, 20 min. AcOH = acetic acid, TBAI = tetrabutylammonium iodide, $Pd(PPh_3)_4$ = tetrakis(triphenylphosphine)palladium(0), TFA = trifluoroacetic acid.

Figure 28 depicts the structures and activities of stilbene and biaryl compounds. RE = relative efficacy of the indicated compound at 1 mM to 100 mM CDCA.

Figure 29 illustrates a summary of structural parameters of compounds of formula (I) that are important for potent FXR activation.

Figure 30 illustrates structures of styrenes and boronic acids used in library construction illustrated in Figure 27.

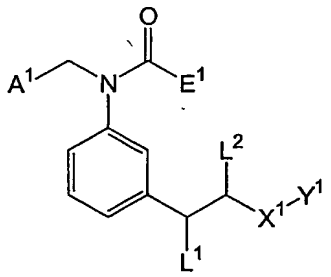
Figure 31 illustrates structures of preferred FXR agonist compounds of formula (II).

Figure 32 illustrates structures of preferred FXR agonist compounds of formula (III).

Figure 33 illustrates structures of preferred FXR agonist compounds of formulas (II), (IV) and (V).

Detailed Description of Preferred Embodiments

The non-steroidal FXR agonists of the present invention are N-aryl-N-arylmethyl amido and ureido compounds represented by the following formula (I):

(I) 

wherein

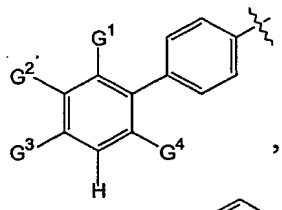
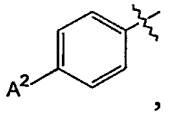
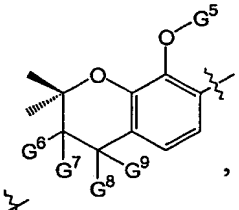
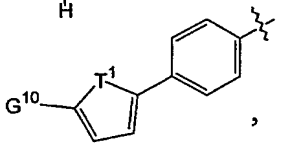
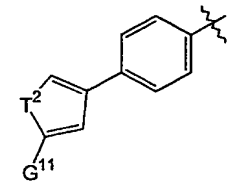
electrophile-derived moiety E^1 is (C_1-C_8)alkyl, cyclohexyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, phenyl, or NH(C_1-C_8)alkyl;

L^1 and L^2 are both H, or together form a pi-bond;

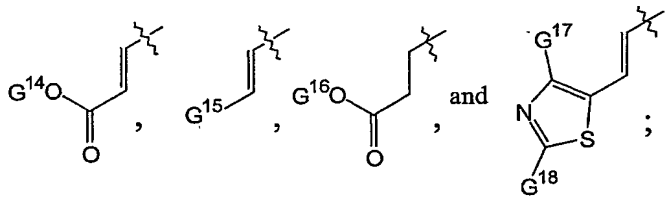
X^1 is C(O), or CH_2;

Y^1 is H, NHZ^1, $NH(Z^2)Z^3$, or OZ^4;

aryl moiety A^1 is selected from the group of radicals consisting of:

 ,  ,  ,  and  ;

A^2 is a radical selected from the group consisting of:



substituent group G^1 is H or OCH_3;

G^2 and G^3 are each independently H, $(C_1$-$C_8)$alkyl, F, Cl, Br, I, OH, $O(C_1$-$C_8)$alkyl, SH, $S(C_1$-$C_8)$alkyl, C(O)H, $C(O)(C_1$-$C_8)$alkyl, $N((C_1$-$C_8)alkyl)_2$, CO_2CH_3, or together form a 5 or 6-member carbocyclic ring or OCH_2O, preferably G^2 and G^3 are each independently H, F, Cl, OCH_3, SCH_3, CH_3, $N(CH_3)_2$, or together form OCH_2O;

G^4 is H or OCH_3;

G^5 is $(C_1$-$C_4)$alkyl or $C(O)(C_1$-$C_8)$alkyl;

G^6 is H, or together with G^8 forms a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

G^7 is H, CH_3, or OZ^5, with the proviso that G^7 is H or CH_3 when G^6 and G^8 together form a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

when G^6 is H, G^8 is H, or together with G^9 forms a moiety selected from the group consisting of $=NOZ^6$ and =O (otherwise, as indicated above, G^8 together with G^6 forms a moiety selected from the group consisting of a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, and a dibromocyclopropyl ring);

G^9 is H, OH, OZ^7, CN, $C(O)O(C_1$-$C_8)$alkyl, SPh, $S(C_1$-$C_8)$alkyl, NHZ^8, $NH(Z^9)Z^{10}$, or together with G^{10} forms a moiety selected from the group consisting of $=NOZ^6$ and =O;

G^{10} and G^{11} are each independently H, $(C_1$-$C_8)$alkyl, SCH_3, $C(O)(C_1$-$C_8)$alkyl, or $C(O)O(C_1$-$C_8)$alkyl; and

G^{12} and G^{13} are each independently H or F;

G^{14} and G^{16} are each independently $(C_1\text{-}C_8)$alkyl, phenyl, or benzyl;

G^{15} is phenyl, $(C_1\text{-}C_8)$alkylphenyl; hydroxyphenyl, $(C_1\text{-}C_8)$alkoxyphenyl, chlorophenyl, bromophenyl, or fluorophenyl; and

G^{17} and G^{18} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl;

T^1 and T^2 are each independently O, S, NH, or $N(C_1\text{-}C_8)$alkyl;

Z^1 is H, phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, or $C(O)NH(C_1\text{-}C_8)$alkyl;

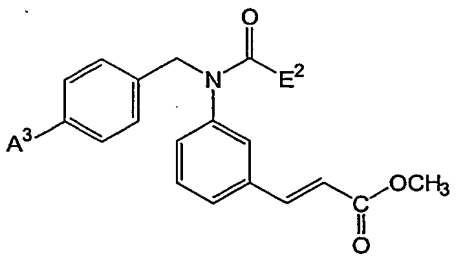
Z^2 and Z^3 are each independently $(C_1\text{-}C_8)$alkyl or together form a $(C_1\text{-}C_8)$cycloalkyl ring;

Z^4, Z^5, Z^6, and $Z^7$are each independently H or an oxygen protecting group, preferably an oxygen protecting group selected from the group consisting of phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, and $C(O)NH(C_1\text{-}C_8)$alkyl;

Z^8 is H, phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, or $C(O)NH(C_1\text{-}C_8)$alkyl;

Z^9 and Z^{10} are each independently $(C_1\text{-}C_8)$alkyl, or together form a $(C_5\text{-}C_8)$cyclic amine ring.

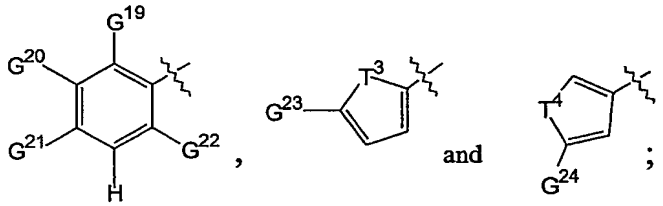
One aspect of the present invention is a biaryl subclass of FXR agonists represented by formula (II):

(II) 

wherein

E^2 is isopropyl or cyclohexyl;

A^3 is an aryl moiety selected from the group consisting of:



G^{19} is H or OCH_3;

G^{20} and G^{21} are each independently H, $(C_1\text{-}C_8)$alkyl, F, Cl, Br, I, OH, $O(C_1\text{-}C_8)$alkyl, SH, $S(C_1\text{-}C_8)$alkyl, C(O)H, $C(O)(C_1\text{-}C_8)$alkyl, $N((C_1\text{-}C_8)alkyl)_2$, CO_2CH_3, or together form a 5 or 6-member carbocyclic ring or OCH_2O; preferably, G^{20} and G^{21} are each independently H, F, Cl, OCH_3, SCH_3, CH_3, $N(CH_3)_2$, or together are OCH_2O;

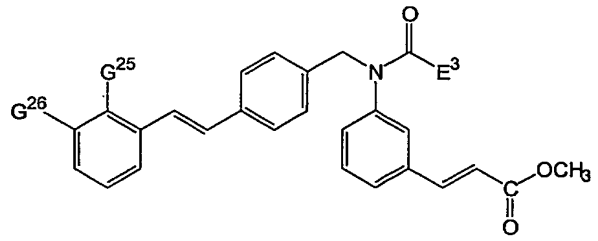
G^{22} is H or OCH_3;

G^{23} and G^{24} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl; and

T^3 and T^4 are each independently O, S, NH, or $N(C_1\text{-}C_8)$alkyl.

Preferred embodiments of FXR agonists of the present invention represented by formula (II) are illustrated in Figure 31 and Figure 33.

Another aspect of the present invention is a stilbene subclass of FXR agonists represented by formula (III):

(III) 

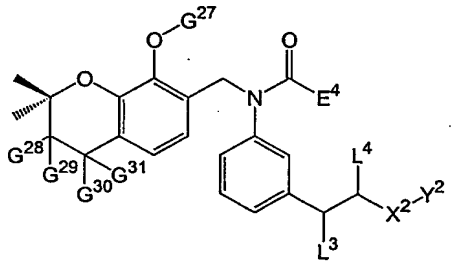
wherein

E^3 is isopropyl or cyclohexyl; and

G^{25} and G^{26} are each independently H or F.

Preferred embodiments of FXR agonists of the present invention represented by formula (III) are illustrated in Figure 32.

Yet another aspect of the present invention is a benzopyran subclass of FXR agonists represented by formula (IV):

(IV) 

wherein

E^4 is $(C_1\text{-}C_8)$alkyl, cyclohexyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, phenyl, or $NH(C_1\text{-}C_8)$alkyl;

L^3 and L^4 are both H, or together form a pi-bond;

X^2 is C(O), or CH_2;

Y^2 is H, NHZ^{11}, $NH(Z^{12})Z^{13}$, or OZ^{14};

G^{27} is $(C_1\text{-}C_4)$alkyl or $C(O)(C_1\text{-}C_8)$alkyl;

G^{28} is H, or together with G^{30} forms a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

G^{29} is H, CH_3, and OZ^{15}, with the proviso that when G^{28} and G^{30} together form a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring, G^{29} is H or CH_3;

when G^{28} is H, G^{30} is H, or together with G^{26} forms a moiety selected from the group consisting of $=NOZ^{16}$ and =O;

G^{31} is H, OH, OZ^{17}, CN, $C(O)O(C_1\text{-}C_8)$alkyl, SPh, $S(C_1\text{-}C_8)$alkyl, NHZ^{18}, $NH(Z^{19})Z^{20}$, or together with G^{30} forms a moiety selected from the group consisting of $=NOZ^{16}$ and =O;

Z^{11} is H, phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, or $C(O)NH(C_1\text{-}C_8)$alkyl;

Z^{12} and Z^{13} are each independently $(C_1\text{-}C_8)$alkyl or together form a $(C_1\text{-}C_8)$cycloalkyl ring;

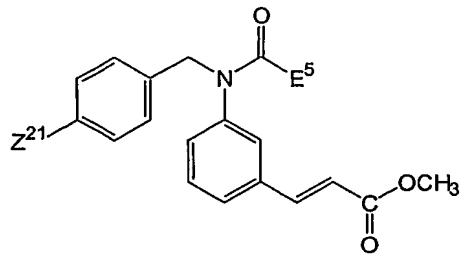
Z^{14}, Z^{15}, Z^{16}, and Z^{17} are each independently H, or an oxygen protecting group, preferably an oxygen protecting group selected from the group consisting of phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, and $C(O)NH(C_1\text{-}C_8)$alkyl;

Z^{18} is H, phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, and $C(O)NH(C_1\text{-}C_8)$alkyl; and

Z^{19} and Z^{20} are each independently $(C_1\text{-}C_8)$alkyl, or together form a $(C_5\text{-}C_8)$cyclic amine ring.

A preferred embodiment of a FXR agonist of the present invention represented by formula (IV) is illustrated in Figure 33.

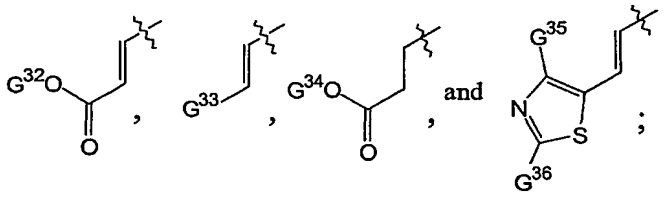
An additional aspect of the present invention is a subclass of FXR agonists represented by formula (V):

(V) 

wherein

E^5 is isopropyl or cyclohexyl;

Z^{21} is a radical selected from the group consisting of:



G^{32} and G^{34} are each independently $(C_1\text{-}C_8)$alkyl, phenyl, or benzyl;

G^{33} is phenyl, $(C_1\text{-}C_8)$alkylphenyl; hydroxyphenyl, $(C_1\text{-}C_8)$alkoxyphenyl, chlorophenyl, bromophenyl, or fluorophenyl; and

G^{35} and G^{36} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl.

Preferred embodiments of FXR agonists of the present invention represented by formula (V) are illustrated in Figure 33.

The FXR agonists represented by formula (I), including compounds of formulas (II), (III), (IV), and (V), are useful as therapeutic agents for the treatment of diseases linked to cholesterol, and bile acid

metabolism and homeostasis. The present FXR agonists are also useful tools for selectively activating FXR *in vivo*.

Initial screening of a diversity-orientated library of 10,000 benzopyran containing small molecules for FXR activation utilizing a cell-based reporter assay led to the identification of several lead compounds possessing low micromolar activity (EC_{50}'s = 5–10 mM). These compounds were systematically optimized employing parallel solution-phase synthesis and solid-phase synthesis to provide compounds that potently activate FXR. Two series of compounds, bearing stilbene or biaryl moieties, contain members that are among the most potent FXR agonists reported to date in cell-based assays. These compounds are useful in studies aimed at further defining the physiological role of FXR and as potential agents for the treatment of diseases linked to cholesterol and bile acid metabolism and homeostasis. In addition to the discovery of potent compounds, the complete investigation of the structure-activity relationship (SAR) of the present agonists provide a valuable knowledge base for development of even more potent FXR agonists. In contrast to the previously reported FXR agonists (see Figure 1), none of the compounds of the present invention contain a carboxylic acid moiety.

Previous screening technologies for identifying small molecule activators of FXR utilized a fluorescence resonance energy transfer (FRET) assay to detect the ligand-dependent recruitment of the coactivator SRC-1 to FXR (Fraser, G. J., et al., *J. Biol. Screen.* **2002**, *7*, 3–10). The association of FXR with a coactivator is a necessary event for transcriptional activation. In the present investigations, however, a cell-based transcription assay was employed in which an FXR responsive promoter is linked to a luciferase reporter as a primary screen. In addition to ensuring that only cell permeable compounds were selected for further optimization, this approach allows for the detection of FXR activation in a natural system (i.e. correct folding of the protein and in the presence of a complete compliment of co-activators and co-repressors) (Xu, L., et al., *Curr. Opin. Gen. Develop.* **1999**, *9*, 140–147; and

Glass, C. K., et al., *Curr. Opin. Cell Biol.* **1997**, *9*, 222–232).

Initial screening of a 10,000-member combinatorial library of benzopyran-based small molecules in this high-throughput, cell-based assay for FXR activation produced several lead compounds whose structures are listed in Figure 3(a) (**4–15**). Guided by the preliminary SAR gained from the evaluation of this initial library, a follow-up focused library of *ca.* 200 benzopyran-based compounds was designed and synthesized on solid support (see Figure 4). A selection of the most active compounds, possessing EC_{50} values between 5 and 10 μM, obtained from this second round of screening is shown in Figure 3(b) (**16–27**). Compounds **26** and **27** proved to be among the most active FXR agonists at this stage and were the subject of further optimization as described below.

With initial lead compounds identified and validated, the stage was set for the systematic optimization of the three regions of the lead structure shown in Figure 5. As described in detail in the following sections, focused libraries were synthesized and screened in the cell-based assay in order to evaluate the structural requirements of each region of the molecule for potent FXR agonism. At this point, parallel solution-phase chemistry was utilized for the construction of additional focused libraries. This shift away from solid-phase chemistry provided maximum flexibility, enabling rapid and systematic optimizations of each region of the lead molecules using relatively small designed libraries.

Evaluation of Benzopyran Region I SAR.

Most of the FXR agonists reported to date including CDCA (**1**), TTNPB (**2**) and GW4064 (**3**) (see Figure 1) contain a carboxylic acid moiety. Based on these prior studies, materials having a variety of substituents within regions I, II and III of structure **26** (Figure 5) were prepared and the SAR of region I was evaluated. Several compounds displaying a carboxylic acid unit in various positions were synthesized (e.g. compounds **28**, **36**, **52**, **54** and **56**, Figure 6) and tested. Surprisingly, none of these compounds exhibited

improved activation of FXR. Interestingly, compound **29**, bearing a *meta* methyl acrylate moiety, was a substantially better activator of FXR than compound **26**.

The preparation of compound **29** is representative of the methods employed to construct these compounds and is described in Figure 7 (see Figures 8-10 for further experimental procedures): aldehyde **59** was selectively methylated (Boger, D. L., et al., *J. Am. Chem. Soc.* **1999**, *121*, 2471–2477). (NaH, MeI), alkylated (2-methyl-3-butyn-2-ol, TFAA, DBU, $CuCl_2$), reduced (Lindlar, H_2) and thermally cyclized to yield benzopyran **60**. Reductive amination of aldehyde **60** with 3-bromoaniline ($NaCNBH_3$) followed by acylation with cyclopropanecarbonyl chloride (C_3H_5COCl, Et_3N) and palladium-mediated Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with methyl acrylate provided compound **29**.

Significantly, the location of the methyl acrylate moiety at the *meta* position led to potent activation of FXR, whereas, compound **53** (Figure 6), which bears a *para* methyl acrylate group did not activate FXR. The additional compounds shown in Figure 6 were synthesized to further examine which functional groups could be tolerated at the *meta* position. From biological screening of these compounds in the cell-based assay described herein, it is likely that the length and rigidity of the tether between the aromatic core and the interacting functionality (either methyl ester or methyl ether) are significant factors for FXR agonist activity. For instance, compounds **41** and **45** possess either too short or too long of a tether, respectively, for potent activity; compounds **35** and **46–49** apparently cannot adopt the correct orientation for potent activation; and compounds **30**, **31**, **34**, **38**, **39**, **40** and **50** apparently do not present active functional groups to the receptor, since they are inactive. Indeed, of all the analogs designed to probe the SAR of region I, only compounds **29** and **33** significantly activated FXR in the cell-based assay. Due to relative ease of synthesis of compound **29**, this analog was chosen as a starting point for the optimization of region II.

Evaluation of Benzopyran Region II SAR.

Figure 11 illustrates the effect of numerous substitution patterns in this region of the molecule (see Figure 12 for a schematic representation of the preparation of these compounds). Only compounds **65** (EC_{50} = 358 nM) and **68** (EC_{50} = ca. 1 μM) were more effective than compound **29** in activating FXR in this series of compounds. Substituted aromatic amide derivatives such as **69–77** were all less active than the parent compound **68**, although they did exhibit significant activity. Alkyl derivatives **78** and **79** were inactive, as were sulfonamide **82**, thiourea **84**, and thioamide **83**, suggesting the importance of N-acylation in region II. These results indicate that region II requires moderately bulky alkyl and cycloalkyl amide moieties for good FXR agonist activity.

Evaluation of Benzopyran Region III SAR.

Region III was optimized after regions I and II were thoroughly examined. Figure 13 shows structures of the compounds prepared for the region III SAR investigation. Figures 14 and 15 schematically illustrate preparation of the compounds. Incorporation of a polar hydrogen-bond donating functional group, such as those present in compounds **86, 93, 94, 98** and **100**, and a hydrogen-bond acceptor group, such as those present in compounds **89, 90, 95, 99** and **101,** did not improve FXR agonist activity over that of the parent compound **68**. Similarly, the addition of a bulky lipophilic group to the benzopyran moiety afforded compounds that only weakly activated FXR. Surprisingly, however, replacement of the double bond in the benzopyran unit by a dichlorocyclopropane unit provided analog **102** (EC_{50} = 333 nM), which was highly active. The synthesis of this potent compound is depicted in Figure 16: benzopyran **103** was cyclopropanated under phase transfer conditions (adogen 464 (cat), NaOH, $CHCl_3$) and converted to the corresponding cinnamate via a Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with methyl acrylate to yield **102**. Replacement of the benzoyl group in region II of compound **102** with the cyclohexanecarbonyl moiety afforded the even more

potent compound **149** (EC_{50} = 188 nM). Compound **149** (EC_{50} = 188 nM) represents a significant improvement in potency over compound **65** (EC_{50} = 358 nM).

The effect of replacing the benzopyran moiety with other ring systems was then examined to gain further insight into the structural requirements for optimal FXR agonist activity. Figure 17 shows a set of compounds in which the benzopyran moiety was replaced with groups of varying molecular diversity (see Figures 18 and 19 for a schematic representation of the synthesis of theses compounds). Results of cell-based reporter assays of the compounds indicated that replacement of the benzopyran with a small aromatic unit generally had a detrimental effect on activity. For instance, compounds **110** and **112–117** in Figure 20(c) were inactive, while compounds **111** and **118** showed only moderate activation of FXR (EC_{50} = 680 nM and 606 nM, respectively). Replacement of the benzopyran with an aromatic ring bearing a substituent at the *para* position produced compounds with improved activity over that of compound **68**. For example, 4-*tert*-butyl cinnamate **105** (EC_{50} = 127 nM), stilbenes **121** and **122** (EC_{50} = 36 and 208 nM, respectively), biaryls **124–127** (EC_{50} = 510, 69, 77, 227 nM, respectively) and aryl thiophenes **128** and **129** (EC_{50} = 206 and 256 nM, respectively) were all potent activators of FXR in the cell-based reporter assay. The synthesis of compound **105** is outlined in Figure 21 (see Figure 22 for the preparation of compounds **121–129**). Acylation of 3-bromoaniline ($C_6H_{11}COCl$, Et_3N) provided cyclohexylamide **131**. Subsequent reaction of **131** under Heck coupling conditions ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with methyl acrylate gave compound **132**. Finally, alkylation (4-bromobenzyl bromide, NaH) of cinnamate **132** followed by a second Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with *tert*-butyl acrylate afforded compound **105**.

This initial survey of the three regions of SAR outlined in Figure 5 led to the identification of a number of potent FXR agonists for further evaluation. The compounds can be classified into four subclasses of

formula (I), supra, namely compounds of formulas (II), (III), (IV), and (V), described above. Benzopyran dichlorocyclopropane compound **149** (EC_{50} = 188 nM) is representative of compounds of formula (IV). Bis-cinnamate compound **105** (EC_{50} = 127 nM) is a member of the formula (V) compounds. Stilbene compound **121** (EC_{50} = 36 nM) and biaryl compound **124** (EC_{50} = 69 nM) are examples of the formula (III) and formula (II) compounds, respectively. Based on data presented in Figures 6, 11, and 13, compound **149** appeared to represent the highest potency compound among the benzopyran compounds of formula (IV). The compounds of formula (V), formula (II), and formula (III) appeared to possess considerable potential for further development and rigorous SAR analysis, the results of which are presented below.

Examination of the bis-cinnamate and related compounds of formula (V).

Similar to the results described above, the *meta* substituted methyl cinnamate moiety on the "right-hand" region of the molecule remained an important factor for good activity in the bis-cinnamate series (see Figure 20(a) and Figure 23). Replacement of the methyl acrylate unit with either a methyl or ethyl allylic ether (compounds **136** and **137**) caused only a slight decrease in activity (EC_{50} = 243 and 220 nM, respectively) compared to compound **105**. A marked decline in potency accompanied substitution of the methyl acrylate by a sterically bulkier ether or ester (**133** and **134**) or amide (**135**). Interestingly, saturation of the acrylate olefin (**139**) afforded only a two-fold decrease in potency, EC_{50} = 274 nM, which supports the hypothesis that conformational rigidity is a factor contributing to, but not essential for, high affinity ligands of FXR. Importantly, compound **139** suggests that the methyl acrylate moiety is not simply functioning as a latent electrophile.

The activity of Region II variants also closely mirrored the preceding data, in that cycloalkyl amides remained the optimal substituents (**105** and **140–142**: EC_{50} = 127–250 nM) in the bis-cinnamate series (see Figure 20(b) and Figure 24). Aromatic and heterocyclic amides as well as alkyl ureas

led to moderate potency (**143–145**: EC_{50} = 205–236 nM), whereas incorporation of a bulky urea such as present in compound **146** rendered compounds of only marginal efficacy.

As mentioned above, replacement of the benzopyran moiety with a benzyl group bearing a *tert*-butyl acrylate moiety in the *para*-position yielded compound **105** with dramatically increased efficacy (EC_{50} = 127 nM). Interestingly, placement of the same *tert*-butyl acrylate group in either the *meta* or *ortho* positions of the aromatic ring (**107** and **109**, Figure 17 and Figure 19 for synthesis) in Region III led to only micromolar potency. Further investigation of the "left-hand" region in this series of compounds demonstrated that a decrease in ester group size yielded a corresponding decrease in efficacy (EC_{50} of *t*-butyl > *i*-propyl > ethyl > methyl; compounds **105**, **150–152**, Figure 20(d) and Figure 25). Similarly, substitution of the ester with either a carboxylic acid or an amide group provided less effective compounds with EC_{50} values in the micromolar range. Compounds in which the *tert*-butyl acrylate moiety was substituted with a methyl or ethyl allylic ether (**156** and **157**) retained considerable potency (EC_{50} = 233 and 198 nM, respectively). The bulkier phenyl allylic ether **158** possessed only micromolar activity, however. In addition, saturation of the acrylate moiety, as in compound **159**, resulted in a two-fold decrease in potency from the parent compound (**105**). Finally, substitution at the *ortho* position of the aromatic ring of the *tert*-butyl acrylate series with oxygenated functionality (e.g., compounds **161–167**, see Figure 20(d) and Figure 26 for synthesis) afforded compounds with very low biological activity.

Construction of Focused Libraries of Biaryl Compounds of Formula (II) and Stilbene Compounds of Formula (III).

In an effort to further optimize the biaryl and stilbene series, a 93-member library of such compounds was constructed employing a split-and-pool solid phase strategy. Individual library members were identified via radio frequency encoding using IRORI™ tags and MarcroKan™

technologies (Nicolaou, K. C., et al., *J. Am. Chem. Soc.* **2000**, *122*, 9939–9953; Nicolaou, K. C., et al., *J. Am. Chem. Soc.* **2000**, *122*, 9954–9967; and Nicolaou, K. C., et al., *J. Am. Chem. Soc.* **2000**, *122*, 9968–9976). As shown in Figure 27, Boc protected cinnamic acid **168** was immobilized on Merrifield resin (Cs_2CO_3) to afford resin **169**. The Boc group of this resin was removed by treatment with 20% TFA in CH_2Cl_2 and the resultant resin-bound amine was reductively alkylated with 4-bromobenzaldehyde ($NaCNBH_3$) to yield amino resin **170**. Resin **170** was acylated with one of three acyl groups to give amide or urea resins **171**. The acylated resins (**171**) were subjected to either Heck coupling ($Pd_2(dba)_3$, P(*o*-tol)$_3$, Et_3N) with thirteen substituted styrenes or Suzuki coupling ($Pd(PPh_3)_4$, Cs_2CO_3) with eighteen boronic acids to yield stilbene resins **172** and biaryl resins **173**, respectively.

In selecting appropriate styrenes and boronic acids as inputs into this combinatorial library, guidance was obtained by initial comparisons of *tert*-butyl stilbene (**123**, EC_{50} = > 1000 nM) to the unsubstituted stilbene **102** (EC_{50} = 36 nM), and biaryl compound **124** (EC_{50} = 510 nM) to **125** (EC_{50} = 69 nM) as shown in Figure 20. Without being bound by theory, it is likely that both the stilbene and the biaryl ligands needed to fit into the same region of space within the receptor site for potent activation. Hence, stilbenes in which the aromatic nucleus is removed two carbon atoms further away from the core of the molecule compared to biaryls, are more potent when adorned with small substituents. In contrast, biaryl compounds are generally more potent when substituted with larger functional groups. Cleavage of resins **172** and **173** with NaOMe yielded methyl acrylates **121**, **125**, **126** and **174–263**. Analysis of the library by liquid chromatograpy / mass spectrometry (LCMS) after purification using preparative thin-layer chromatography (PTLC), indicated that the average purity of these compounds was generally greater than 95%.

Screening of this compound library in the cell-based reporter assay led to some intriguing results as summarized in Figure 28. For example, in both the stilbene and biaryl series, analogs bearing the cyclohexyl amide

moiety were generally the most potent followed by those bearing the isopropyl amide or isopropyl urea groups. Stilbenes bearing relatively small substituents were more potent than those carrying larger substituents. For instance, unsubstituted stilbene **121** and mono-fluoro stilbenes **192**, **200**, and **203** were among the most active, while the mono-methyl derivative **174** and tri-methyl derivative **195** were among the least active. Interestingly, the heterocyclic compounds **207** and **210** retained good potency (EC_{50} = 309 and 227 nM, respectively).

In the biaryl series, compounds which presented bulkier substituents at the terminus of the structure were the most active, particularly compounds **259** (EC_{50} = 25 nM) and **244** (EC_{50} = 38 nM). Overall, most of the compounds synthesized in this follow-up study were efficient activators of FXR, providing further support for the structural requirements for the FXR binding pocket described above. This model provides a solid basis for further development of FXR activators.

A summary of the molecular requirements of compounds of formula (I) that are important for potent FXR activation is shown in Figure 29. In region I, the presence of the *meta* methyl acrylate unit or an allylic methyl ether is important for potent activation, as only a few modifications retained good activity. The most potent compounds possessed a cycloalkylamide group in region II. Finally, region III is the most tolerant toward structural variations and several structural elements were found to provide a good fit within the pocket of the receptor.

In order to determine how selectively the compounds of the present invention activated FXR, some of the most active compounds were screened against a panel of nuclear receptors to look for cross-activation. The lead compounds were analyzed for their ability to modulate the activity of the following nuclear receptors: RXRαa, PPARα, PPARγ, PPARδ, PXR, SXR, LXRα, TRβ, RARβ, CARR, ERR3, VDR. Most of these compounds were found to be highly selective, activating only FXR. Notably, however,

compound **149** also potently activated SXR (FXR: EC_{50} = 188 nM, SXR: EC_{50} = 77 nM). This result may ultimately lead to compounds having utility in the treatment of diseases linked to the accumulation of toxic bile acids (Willson, T. M., et al., *J. Lipid. Res.* **2002**, *43*, 359–364; and Wen, X., et al., *Proc. Nat. Acad. Sci.* **2001**, *98*, 3375–3380).

General Techniques

Reagents and resins were purchased at highest commercial quality and used without further purification, unless otherwise stated. Anhydrous solvents were obtained by passing them through commercially available alumina column. All reactions were carried out under an argon atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. Solution phase reactions were monitored by thin layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and 7% ethanolic phosphomolybdic acid or *p*-anisaldehyde solution and heat as developing agents. E. Merck silica gel (60, particle size 0.040-0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25 mm E. Merck silica gel plates (60F-254). All final products cleaved from solid support were characterized by LCMS. NMR spectra were recorded on Bruker DRX-600, AMX-500 or AMX-400 instruments and calibrated using residual undeuterated solvent as an internal reference. High resolution mass spectra (HRMS) were recorded on a VG ZAB-ZSE mass spectrometer under MALDI-FTMS conditions with NBA as the matrix. Representative procedures for each region of SAR and the final combinatorial library synthesized are provided below. Compounds were screened for their ability to activate FXR expression *in vivo*. Solutions of the compounds at varying concentrations were added to cultures of cells that were cotransfected with human FRX gene linked to a luciferase reporter gene (see Downes, *et al.*, *Mol. Cell*, 2003; 11: 1079-1092; Nicolaou *et al. Org. Biomol. Chem.*, 2003; 1: 908-920). Expression of FXR in the cells was designed to drive luciferase expression. FXR activation

was determined by photometric measurement of luciferase luminescence. This approach allows for the detection of FXR activation in a natural system, i.e., correct folding of the protein and in the presence of a complete compliment of coactivators and corepressors (see Xu *et al. Curr. Opin. Gen. Dev.*, 1999; 9:140-147; and Glass *et al. Curr. Opin. Cell Biol.*, 1997; 9:222-232).

Representative procedure for synthesis Region I/II analogues; Synthesis of acrylate 29 (Figure 7):

To a solution of aldehyde **60** (50.0 mg, 0.229 mmol, 1.0 equivalent) in THF (1.0 mL) at 25°C was added 3-bromoaniline (59.0 mg, 0.344 mmol, 1.5 equivalents) and the reaction mixture was heated to 70°C. The solution was stirred for 4 hours and cooled to ambient temperature. To the resulting mixture was added methanol (0.2 mL) and $NaCNBH_3$ (28.8 mg, 0.458 mmol, 2.0 equivalents) and heated to 70°C for 4 hours. The reaction mixture was then cooled and quenched with brine (5 mL). The reaction mixture was then concentrated and extracted with EtOAc (3 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated and used without further purification (90% yield by crude ^1H NMR analysis). To a solution of the resulting secondary amine (0.206 mmol, 1.0 equivalent) in CH_2Cl_2 (1.0 mL) was added triethylamine (0.038 mL, 0.268 mmol, 1.3 equivalents), 4-DMAP (2.6 mg, 0.021 mmol, 0.1 equivalent), and cyclopropanecarbonyl chloride (28.0 mg, 0.268 mmol, 1.3 equivalents). The reaction mixture was stirred at 25°C for 12 hours and quenched with the addition of brine (5 mL). The aqueous phase was then extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated and used without further purification (95% yield by crude ^1H NMR analysis). To the resulting amide (0.196 mmol, 1.0 equivalent) in *N,N*-dimethylformamide (1.0 mL) was added triethylamine (0.137 mL, 0.980 mmol, 5.0 equivalents), methyl acrylate (0.071 mL, 0.784 mmol, 4.0 equivalents), tri-*o*-tolylphosphine (30.0 mg, 0.098 mmol, 0.5 equivalent), and tris(dibenzylidineacetone)dipalladium(0) (35.9 mg, 0.039 mmol, 0.2

equivalent) sequentially and heated to 90°C. The reaction mixture was stirred for 24 hours and then cooled to ambient temperature. The reaction mixture was then diluted with EtOAc (10 mL) and washed with water (3 x 5 mL) and brine (1 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (silica, 0 (30% EtOAc in hexanes) to afford **29** (70.1 mg, 80%). **29**: R_f = 0.42 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2943, 1720, 1642, 1596, 1443, 1414, 1314, 1267, 1202 cm^{-1}; HRMS calcd for $C_{27}H_{29}NO_5$ $[M + H^+]$ 448.2118, found 448.2117.

Representative procedure for synthesis Region III benzopyran containing analogues; Synthesis of dichlorocyclopropane 102 (Figure 16):

To a solution of **103** (50.0 mg, 0.089 mmol, 1.0 equivalent) in $CHCl_3$ (2.0 mL) at 25°C was added NaOH (2.0 N, 0.3 mL) and adogen 464 (5.0 mg, ca. 0.1 equivalent). The resulting reaction mixture was stirred for 6 hours and quenched with water (5 mL). The aqueous phase was then extracted with CH_2Cl_2 (3 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered and concentrated, and used without further purification (85% yield by crude ^1H NMR analysis). To the resulting dichlorocyclopropane (0.073 mmol, 1.0 equivalent) in *N,N*-dimethylformamide (2.0 mL) was added triethylamine (0.051 mL, 0.366 mmol, 5.0 equivalents), methyl acrylate (0.026 mL, 0.292 mmol, 4.0 equivalents), tri-*o*-tolylphosphine (11.1 mg, 0.037 mmol, 0.5 equivalent), and tris(dibenzylidineacetone)dipalladium(0) (13.4 mg, 0.015 mmol, 0.2 equivalent) sequentially and heated to 90°C. The reaction mixture was stirred for 24 hours and then cooled to ambient temperature. The reaction mixture was then diluted with EtOAc (10 mL) and washed with water (3 x 5 mL) and brine (1 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (silica, 0 (30% EtOAc in hexanes) to afford **102** (30.9 mg, 75%).

102: R_f = 0.38 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2943, 1719, 1640, 1590, 1443, 1378, 1314, 1226, 1161 cm^{-1}; HRMS calcd for

$C_{31}H_{29}Cl_2NO_5$ [M + Na^+] 588.1315, found 588.1323.

Representative procedure for synthesis Region III non-benzopyran containing analogues; Synthesis of bis-cinnamate 105 (Figure 21):

To a solution of 3-bromoaniline (**130**, 60.0 mg, 0.349 mmol, 1.0 equivalent) in CH_2Cl_2 (1.0 mL) at 25°C was added triethylamine (0.064 mL, 0.453 mmol, 1.3 equivalents), 4-DMAP (2.1 mg, 0.017 mmol, 0.05 equivalent), and cyclohexanecarbonyl chloride (56.3 mg, 0.384 mmol, 1.1 equivalents). The reaction mixture was stirred for 3 hours and quenched with brine (5 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 5 mL) and subsequently dried over $MgSO_4$, filtered, concentrated to afford amide **131** (95% yield by crude ^1H NMR analysis) which was utilized without further purification. To a solution of amide **131** (0.332 mmol, 1.0 equivalent) in *N,N*-dimethylformamide (2.0 mL) was added triethylamine (0.232 mL, 1.66 mmol, 5.0 equivalents), methyl acrylate (0.119 mL, 1.33 mmol, 4.0 equivalents), tri-*o*-tolylphosphine (60.8 mg, 0.199 mmol, 0.6 equivalent), and tris(dibenzylidineacetone)dipalladium(0) (60.8 mg, 0.066 mmol, 0.2 equivalent) and heated to 90°C. The reaction mixture was stirred for 24 hours and then cooled to ambient temperature. The reaction mixture was then diluted with EtOAc (15 mL) and washed with water (3 x 5 mL) and brine (1 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (silica, 0 (50% EtOAc in hexanes) to afford **132** (71.6 mg, 75%). To a solution of acrylate **132** (60.0 mg, 0.209 mmol, 1.0 equivalent) in THF (1.0 mL) at 0°C was added NaH (9.2 mg, 0.230 mmol, 60% dispersion in mineral oil, 1.1 equivalents) followed by 4-bromobenzyl bromide (67.9 mg, 0.272 mmol, 1.3 equivalents). The reaction mixture was stirred for 2 hours and quenched with saturated NH_4Cl (5 mL). The aqueous phase was extracted with EtOAc (3 x 5 mL) and the combined organic phase was dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (silica, 0 (30% EtOAc in hexanes) to afford **112** (85.8

mg, 90%). To a solution of amide **112** (50.0 mg, 0.110 mmol, 1.0 equivalent) in *N,N*-dimethylformamide (2.0 mL) was added triethylamine (0.077 mL, 0.550 mmol, 5.0 equivalents), *tert*-butyl acrylate (0.064 mL, 0.440 mmol, 4.0 equivalents), tri-*o*-tolylphosphine (5.0 mg, 0.017 mmol, 0.15 equivalent), and tris(dibenzylidineacetone)dipalladium(0) (5.0 mg, 0.006 mmol, 0.05 equivalent) and heated to 90°C. The reaction mixture was stirred for 12 hours and then cooled to ambient temperature. The reaction mixture was then diluted with EtOAc (5 mL) and washed with water (3 x 5 mL) and brine (1 x 5 mL). The combined organic phase was dried over $MgSO_4$, filtered, concentrated and purified by column chromatography (silica, 0 (50% EtOAc in hexanes) to afford **105** (fexaramate, 41.5 mg, 75%), a representative member of the formula (V) compounds.

105: R_f = 0.40 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2977, 2931, 2855, 1713, 1640, 1483, 1446, 1393, 1367, 1323, 1279, 1209 cm^{-1}; HRMS calcd for $C_{31}H_{37}NO_5$ $[M + H^+]$ 504.2744, found 504.2764.

General procedure for the solid phase synthesis of 93-membered library of biaryl and stilbene cinnamates (formula (II) compounds 125, 126, and 213-264, and formula (III) compounds 121, and 174-212, Figure 27):

This library was constructed *via* directed split-and-pool techniques using IRORI MacroKans™. The microreactors were initially filled with commercially available Merrifield resin (110 mg, 0.91 mmol/g). After encoding, all 93 microreactors were suspended in *N,N*-dimethylformamide (900 mL) and treated with Boc-protected cinnamic acid **168** (4.94 g, 18.8 mmol, 2.0 equivalents), $CsCO_3$ (6.13 g, 18.8 mmol, 2.0 equivalents) and TBAI (1.73 g, 4.7 mmol, 0.5 equivalent), and heated to 55°C. After 24 hours, the reaction mixture was cooled to ambient temperature and the reaction solvent was decanted prior to washing the microreactors with MeOH (3 x 500 mL), CH_2Cl_2 (3 x 500 mL), and Et_2O (3 x 500 mL). Subsequently all microreactors were pooled and suspended in CH_2Cl_2 (1000 mL) at 25°C and treated with

trifluoroacetic acid (200 mL). After 1 hour, the reaction mixture was quenched with Et_3N (200 mL) and the reaction solvent was decanted prior to washing the microreactors with MeOH (3 x 500 mL), CH_2Cl_2 (3 x 500 mL), and Et_2O (3 x 500 mL). The microreactors were then pooled and resuspended in THF:MeOH (2:1, 1000 mL) at 25°C and treated with 4-bromobenzaldehyde (17.4 g, 94.0 mmol, 10.0 equivalents) and acetic acid (30 mg, 0.47 mmol, 0.05 equivalent). After 1 hour, $NaCNBH_3$ (4.72 g, 75.2 mmol, 8.0 equivalents) was added and the resulting reaction was stirred a further 2 hours. The reaction solvent was then decanted and the microreactors were washed with MeOH (3 x 500 mL), CH_2Cl_2 (3 x 500 mL), and Et_2O (3 x 500 mL).

At this point the microreactors were sorted into one of three reaction vessels and subjected to one of two acylation protocols. The microreactors of two of the reaction vessels were suspended in CH_2Cl_2 (500 mL) at 25°C and treated with either cyclohexanecarbonyl or isobutyryl chloride (94.0 mmol, 30.0 equivalents), Et_3N (17.4 mL, 124 mmol, 40.0 equivalents), and 4-DMAP (380 mg, 3.1 mmol, 1.0 equivalent) and stirred for 12 hours. The microreactors of the remaining reaction vessel were suspended in *N,N*-dimethylformamide (350 mL) and treated with isopropyl isocyanate (8.0 g, 94.0 mmol, 30.0 equivalents), Et_3N (17.4 mL, 124 mmol, 40.0 equivalents), and 4-DMAP (380 mg, 3.1 mmol, 1.0 equivalent), heated to 60°C and stirred for 60 hours. The microreactors were then cooled and the reaction solvent was decanted prior to washing the microreactors with MeOH (3 x 500 mL), CH_2Cl_2 (3 x 500 mL), and Et_2O (3 x 500 mL). The microreactors were then sorted into one of 31 reaction vessels to be treated with either one of 13 commercially available styrenes or one of 18 commercially available boronic acids. For Heck couplings: The microreactors were suspended in *N,N*-dimethylformamide (100 mL) and treated with a stryrene (2.4 mmol, 8.0 equivalents, see Figure 30 for the identities of styrenes), Et_3N (0.42 mL, 3.0 mmol, 10.0 equivalents), tri-*o*-tolylphosphine (138 mg, 0.45 mmol, 1.5 equivalents), and tris(dibenzylidineacetone)dipalladium(0) (138 mg, 0.15 mmol, 0.5 equivalent)

and heated to 90°C for a period of 48 hours. For Suzuki couplings: The microreactors were suspended in *N,N*-dimethylformamide (100 mL) and treated with a boronic acid (2.4 mmol, 8.0 equivalents, see Figure 30 for the identities of boronic acids), $CsCO_3$ (293 mg, 0.9 mmol, 3.0 equivalents), and tetrakis(triphenylphosphine)palladium(0) (173 mg, 0.15 mmol, 0.5 equivalent) and heated to 90°C for a period of 24 hours. The microreactors were then pooled and the reaction solvent was decanted prior to washing the microreactors with MeOH (3 x 500 mL), CH_2Cl_2 (3 x 500 mL), and Et_2O (3 x 500 mL). Finally, each microreactor was sorted into an individual reaction vessel and cleaved upon suspension in Et_2O and subsequent treatment with a solution of NaOMe in MeOH (approx. 10 equivalents) at 25°C for a period of 20 min. The reactions were quenched with brine, extracted with Et_2O, concentrated and each compound was purified by preparatory thin layer chromatography (PTLC). Each compound was analyzed using LCMS which gave an average purity of 95% for the library and 93/93 parent mass peaks found.

Full characterization of representative members of the four classes of potent, selective FXR agonists (Figure 2):

Compounds of Formula (II):

125: R_f = 0.33 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 3025, 2926, 2854, 1714, 1643, 1597, 1580, 1488, 1435, 1394, 1359, 1318, 1269, 1231, 1202 cm^{-1}; HRMS calcd for $C_{31}H_{33}NO_3S$ $[M + Na^+]$ 522.2073, found 522.2053.

128: R_f = 0.43 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 3058, 2927, 2854, 1715, 1642, 1598, 1588, 1483, 1435, 1398, 1318, 1271, 1230, 1201 cm^{-1}; HRMS calcd for $C_{29}H_{31}NO_3S$ $[M + Na^+]$ 496.1917, found 496.1924.

244: R_f = 0.25 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2930, 2855, 1716, 1652, 1599, 1579, 1504, 1486, 1445, 1398, 1318, 1226 cm^{-1}; HRMS calcd for $C_{31}H_{31}NO_5$ [M + H^+] 498.2275, found 498.2269.

259: R_f = 0.27 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2928, 1716, 1646, 1609,1579, 1539, 1504, 1446, 1396, 1357, 1319, 1268 cm^{-1}; HRMS calcd for $C_{32}H_{36}N_2O_3$ [M + H^+] 496.2720, found 496.2715.

Compounds of Formula (III):

121: R_f = 0.35 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2928, 2854, 1717, 1646, 1597, 1578, 1508, 1489, 1448, 1397, 1318, 1268, 1200 cm^{-1}; HRMS calcd for $C_{32}H_{33}NO_3$ [M + H^+] 480.2533, found 480.2534.

192: R_f = 0.35 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2928, 2854, 1716, 1652, 1508, 1485, 1449, 1397, 1318, 1268, 1231, 1200 cm^{-1}; HRMS calcd for $C_{32}H_{32}FNO_3$ [M + H^+] 498.2439, found 498.2450.

Compound of Formula (IV):

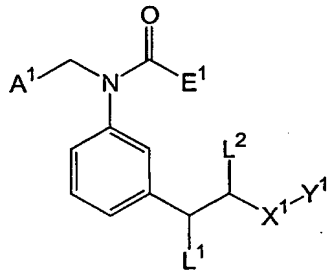
149: R_f = 0.25 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2932, 1720, 1642, 1580, 1454, 1399, 1341, 1318, 1269, 1202 cm^{-1}; HRMS calcd for $C_{31}H_{35}Cl_2NO_5$ [M + Na^+] 594.1784, found 594.1790.

Compound of Formula (V):

210: R_f = 0.10 (silica, 25% ethyl acetate in hexane); FT-IR (neat) ν_{max} 2930, 1715, 1644, 1530, 1446, 1398, 1318, 1174 cm^{-1}; HRMS calcd for $C_{30}H_{32}N_2O_3S$ [M + H^+] 501.2206, found 501.2202.

We claim:

1. A non-steroidal farnesoid X receptor (FXR) agonist having the chemical structure represented by the following formula (I):

(I) 

wherein

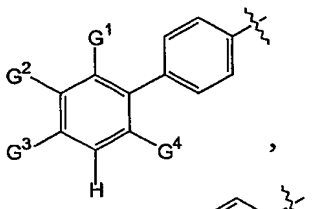
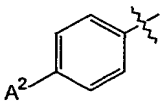
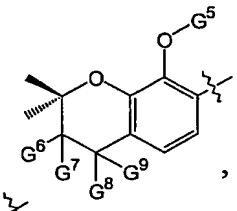
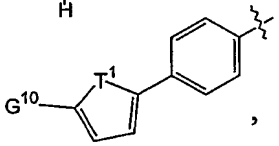
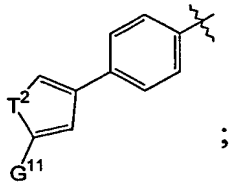
electrophile-derived moiety E^1 is $(C_1\text{-}C_8)$alkyl, cyclohexyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, phenyl, or $NH(C_1\text{-}C_8)$alkyl;

L^1 and L^2 are both H, or together form a pi-bond;

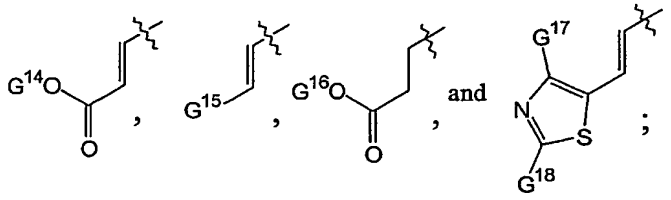
X^1 is C(O), or CH_2;

Y^1 is H, NHZ^1, $NH(Z^2)Z^3$, or OZ^4;

aryl moiety A^1 is selected from the group of radicals consisting of:

 ,  ,  ,  , and  ;

A^2 is a radical selected from the group consisting of:



substituent group G^1 is H or OCH_3;

G^2 and G^3 are each independently H, $(C_1\text{-}C_8)$alkyl, F, Cl, Br, I, OH, $O(C_1\text{-}C_8)$alkyl, SH, $S(C_1\text{-}C_8)$alkyl, C(O)H, $C(O)(C_1\text{-}C_8)$alkyl, $N((C_1\text{-}C_8)alkyl)_2$, CO_2CH_3, or together form a 5 or 6-member carbocyclic ring or OCH_2O;

G^4 is H or OCH_3;

G^5 is $(C_1\text{-}C_4)$alkyl or $C(O)(C_1\text{-}C_8)$alkyl;

G^6 is H, or together with G^8 forms a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

G^7 is H, CH_3, or OZ^5, with the proviso that G^7 is H or CH_3 when G^6 and G^8 together form a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

when G^6 is H, G^8 is H, or together with G^9 forms a moiety selected from the group consisting of $=NOZ^6$ and =O;

G^9 is H, OH, OZ^7, CN, $C(O)O(C_1\text{-}C_8)$alkyl, SPh, $S(C_1\text{-}C_8)$alkyl, NHZ^8, $NH(Z^9)Z^{10}$, or together with G^{10} forms a moiety selected from the group consisting of $=NOZ^6$ and =O;

G^{10} and G^{11} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl; and

G^{12} and G^{13} are each independently H or F;

G^{14} and G^{16} are each independently $(C_1\text{-}C_8)$alkyl, phenyl, or benzyl;

G^{15} is phenyl, $(C_1\text{-}C_8)$alkylphenyl; hydroxyphenyl, $(C_1\text{-}C_8)$alkoxyphenyl, chlorophenyl, bromophenyl, or fluorophenyl; and

G^{17} and G^{18} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl;

T^1 and T^2 are each independently O, S, NH, or N(C_1-C_8)alkyl;

Z^1 is H, phenyl, (C_1-C_8)alkyl, benzyl, C(O)Ph, C(O)(C_1-C_8)alkyl, $C(O)OCH_2Ph$, or C(O)NH(C_1-C_8)alkyl;

Z^2 and Z^3 are each independently (C_1-C_8)alkyl or together form a (C_1-C_8)cycloalkyl ring;

Z^4, Z^5, Z^6, and $Z^7$are each independently H or an oxygen protecting group;

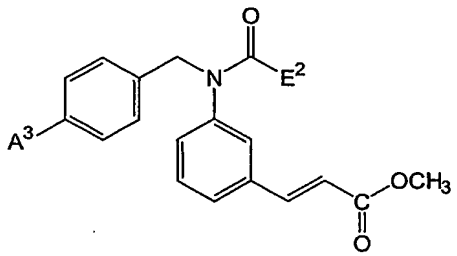
Z^8 is H, phenyl, (C_1-C_8)alkyl, benzyl, C(O)Ph, C(O)(C_1-C_8)alkyl, $C(O)OCH_2Ph$, or C(O)NH(C_1-C_8)alkyl;

Z^9 and Z^{10} are each independently (C_1-C_8)alkyl, or together form a (C_5-C_8)cyclic amine ring.

2. The non-steroidal FXR agonist of claim 1 wherein Z^4, Z^5, Z^6, and Z^7 are each independently H, or an oxygen protecting group selected from the group consisting of phenyl, (C_1-C_8)alkyl, benzyl, C(O)Ph, C(O)(C_1-C_8)alkyl, $C(O)OCH_2Ph$, and C(O)NH(C_1-C_8)alkyl.

3. The non-steroidal FXR agonist of claim 1 represented by formula (II):

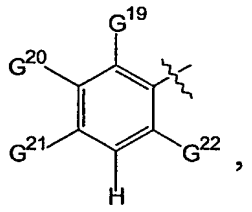
(II)

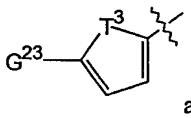


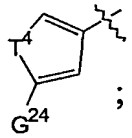
wherein

E^2 is isopropyl or cyclohexyl;

A^3 is an aryl moiety selected from the group consisting of:



,


and


;

G^{19} is H or OCH_3;

G^{20} and G^{21} are each independently H, $(C_1\text{-}C_8)$alkyl, F, Cl, Br, I, OH, $O(C_1\text{-}C_8)$alkyl, SH, $S(C_1\text{-}C_8)$alkyl, C(O)H, $C(O)(C_1\text{-}C_8)$alkyl, $N((C_1\text{-}C_8)alkyl)_2$, CO_2CH_3, or together form a 5 or 6-member carbocyclic ring or OCH_2O;

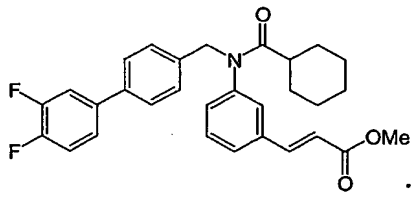
G^{22} is H or OCH_3;

G^{23} and G^{24} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl; and

T^3 and T^4 are each independently O, S, NH, or $N(C_1\text{-}C_8)$alkyl.

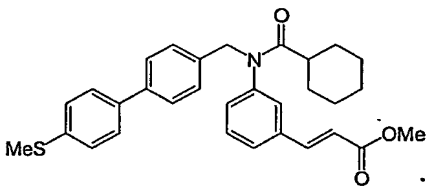
4. The non-steroidal FXR agonist of claim 3 wherein G^{20} and G^{21} are each independently H, F, Cl, OCH_3, SCH_3, CH_3, $N(CH_3)_2$, or together are OCH_2O.

5. The non-steroidal FXR agonist of claim 3 represented by the formula:

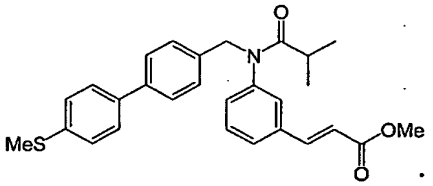


.

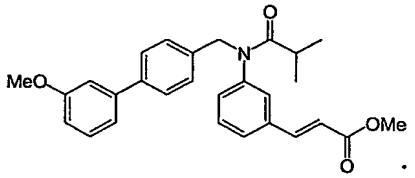
6. The non-steroidal FXR agonist of claim 3 represented by the formula:



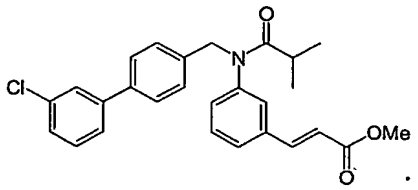
7. The non-steroidal FXR agonist of claim 3 represented by the formula:



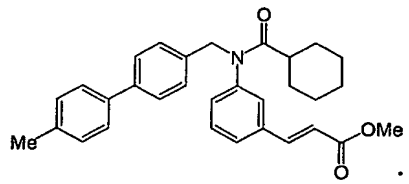
8. The non-steroidal FXR agonist of claim 3 represented by the formula:



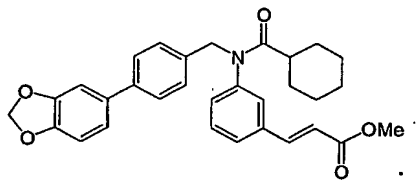
9. The non-steroidal FXR agonist of claim 3 represented by the formula:



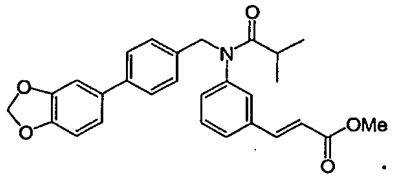
10. The non-steroidal FXR agonist of claim 3 represented by the formula:



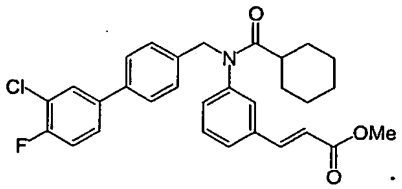
11. The non-steroidal FXR agonist of claim 3 represented by the formula:



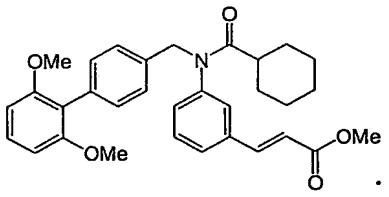
12. The non-steroidal FXR agonist of claim 3 represented by the formula:



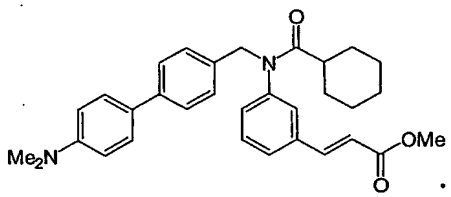
13. The non-steroidal FXR agonist of claim 3 represented by the formula:



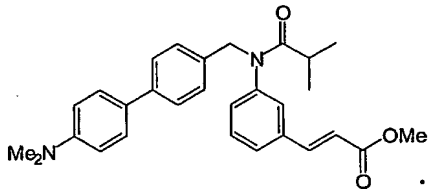
14. The non-steroidal FXR agonist of claim 3 represented by the formula:



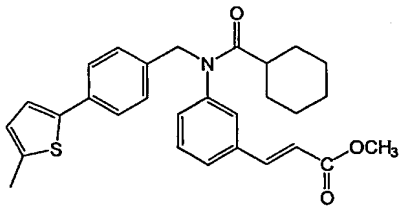
15. The non-steroidal FXR agonist of claim 3 represented by the formula:



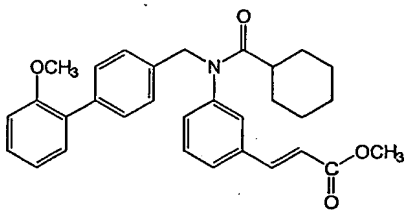
16. The non-steroidal FXR agonist of claim 3 represented by the formula:



17. The non-steroidal FXR agonist of claim 3 represented by the formula:

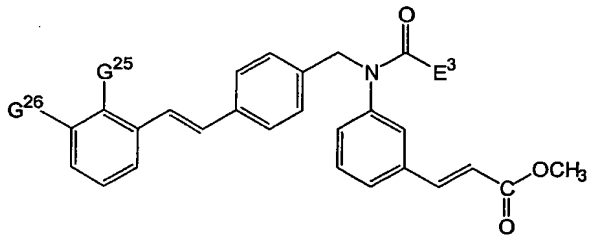


18. The non-steroidal FXR agonist of claim 3 represented by the formula:



19. The non-steroidal FXR agonist of claim 1 represented by formula (III):

(III)

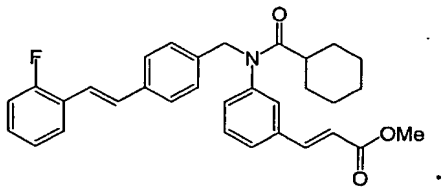


wherein

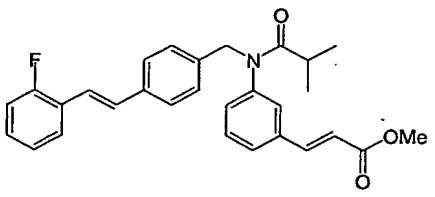
E^3 is isopropyl or cyclohexyl; and

G^{25} and G^{26} are each independently H or F.

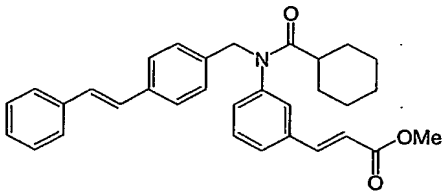
20. The non-steroidal FXR agonist of claim 19 represented by the formula:



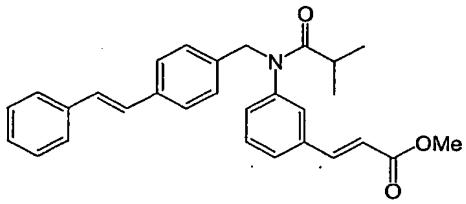
21. The non-steroidal FXR agonist of claim 19 represented by the formula:



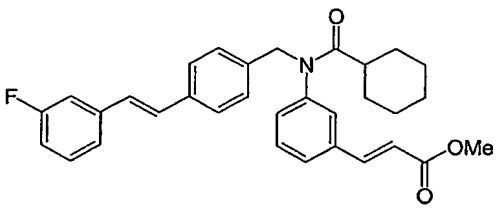
22. The non-steroidal FXR agonist of claim 19 represented by the formula:



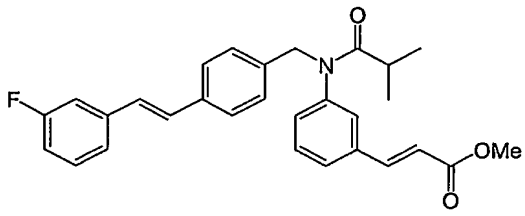
23. The non-steroidal FXR agonist of claim 19 represented by the formula:



24. The non-steroidal FXR agonist of claim 19 represented by the formula:

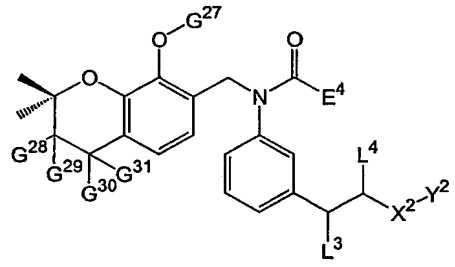


25. The non-steroidal FXR agonist of claim 19 represented by the formula:



26. The non-steroidal FXR agonist of claim 1 represented by formula (IV):

(IV)



wherein

E^4 is $(C_1\text{-}C_8)$alkyl, cyclohexyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, phenyl, or $NH(C_1\text{-}C_8)$alkyl;

L^3 and L^4 are both H, or together form a pi-bond;

X^2 is C(O), or CH_2;

Y^2 is H, NHZ^{11}, $NH(Z^{12})Z^{13}$, or OZ^{14};

G^{27} is $(C_1\text{-}C_4)$alkyl or $C(O)(C_1\text{-}C_8)$alkyl;

G^{28} is H, or together with G^{30} forms a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring;

G^{29} is H, CH_3, and OZ^{15}, with the proviso that when G^{28} and G^{30} together form a pi-bond, an epoxide, a cyclopropyl ring, a dichlorocyclopropyl ring, or a dibromocyclopropyl ring, G^{29} is H or CH_3;

when G^{28} is H, G^{30} is H, or together with G^{26} forms a moiety selected from the group consisting of $=NOZ^{16}$ and =O;

G^{31} is H, OH, OZ^{17}, CN, $C(O)O(C_1\text{-}C_8)$alkyl, SPh, $S(C_1\text{-}C_8)$alkyl,

NHZ^{18}, $NH(Z^{19})Z^{20}$, or together with G^{30} forms a moiety selected from the group consisting of $=NOZ^{16}$ and =O;

Z^{11} is H, phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, or $C(O)NH(C_1\text{-}C_8)$alkyl;

Z^{12} and Z^{13} are each independently $(C_1\text{-}C_8)$alkyl or together form a $(C_1\text{-}C_8)$cycloalkyl ring;

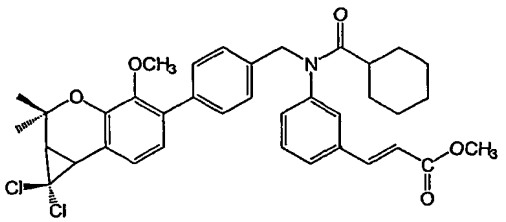
Z^{14}, Z^{15}, Z^{16}, and Z^{17} are each independently H, or an oxygen protecting group;

Z^{18} is H, phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, and $C(O)NH(C_1\text{-}C_8)$alkyl; and

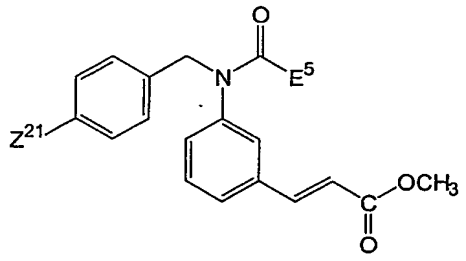
Z^{19} and Z^{20} are each independently $(C_1\text{-}C_8)$alkyl, or together form a $(C_5\text{-}C_8)$cyclic amine ring.

27. The non-steroidal FXR agonist of claim 26 wherein Z^{14}, Z^{15}, Z^{16}, and Z^{17} are each independently H, or an oxygen protecting group selected from the group consisting of phenyl, $(C_1\text{-}C_8)$alkyl, benzyl, C(O)Ph, $C(O)(C_1\text{-}C_8)$alkyl, $C(O)OCH_2Ph$, and $C(O)NH(C_1\text{-}C_8)$alkyl.

28. The non-steroidal FXR agonist of claim 26 represented by the formula:



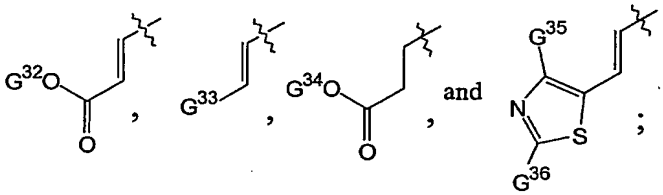
29. The non-steroidal FXR agonist of claim 1 represented by formula (V):

(V) 

wherein

E^5 is isopropyl or cyclohexyl;

Z^{21} is a radical selected from the group consisting of:

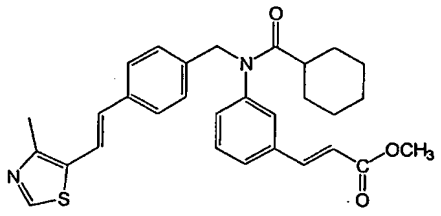


G^{32} and G^{34} are each independently $(C_1\text{-}C_8)$alkyl, phenyl, or benzyl;

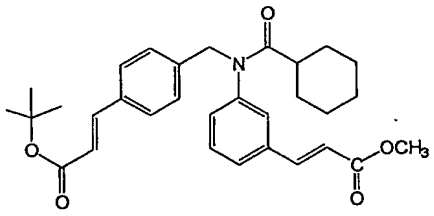
G^{33} is phenyl, $(C_1\text{-}C_8)$alkylphenyl; hydroxyphenyl, $(C_1\text{-}C_8)$alkoxyphenyl, chlorophenyl, bromophenyl, or fluorophenyl; and

G^{35} and G^{36} are each independently H, $(C_1\text{-}C_8)$alkyl, SCH_3, $C(O)(C_1\text{-}C_8)$alkyl, or $C(O)O(C_1\text{-}C_8)$alkyl.

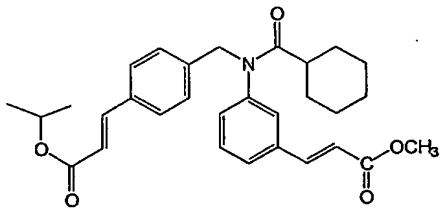
30. The non-steroidal FXR agonist of claim 29 represented by the formula:

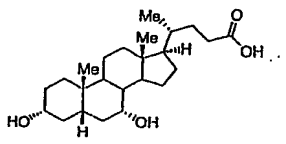


31. The non-steroidal FXR agonist of claim 29 represented by the formula:

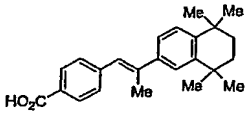


32. The non-steroidal FXR agonist of claim 29 represented by the formula:

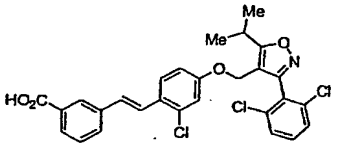




1: CDCA
(low affinity endogenous agonist)

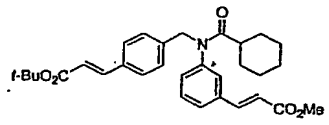


2: TTNPB
(low affinity agonist; EC_{50} > 1μM)

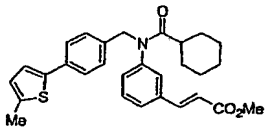


3: GW 4084
(high affinity agonist; EC_{50} = 80 nM)[a]

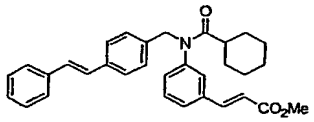
Figure 1



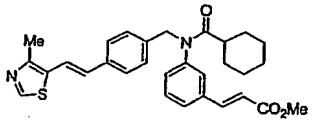
105 (EC_{50} = 127 nM, RE^a = 2.12)
fexaramate



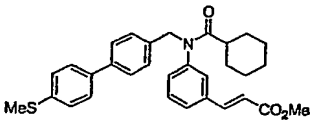
128 (EC_{50} = 206 nM, RE^a = 1.78)



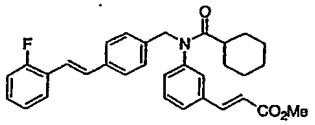
121 (EC_{50} = 38 nM, RE^a = 1.55)
fexarene



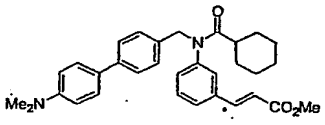
210 (EC_{50} = 227 nM, RE^a = 0.53)



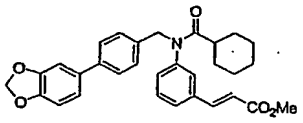
125 (EC_{50} = 69 nM, RE^a = 1.74)



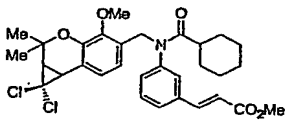
192 (EC_{50} = 64 nM, RE^a = 1.41)



259 (EC_{50} = 25 nM, RE^a = 1.72)
fexaramine



244 (EC_{50} = 38 nM, RE^a = 1.90)
fexarine



149 (EC_{50} = 188 nM, RE^a = 0.50)
fexerchloramide

Figure 2

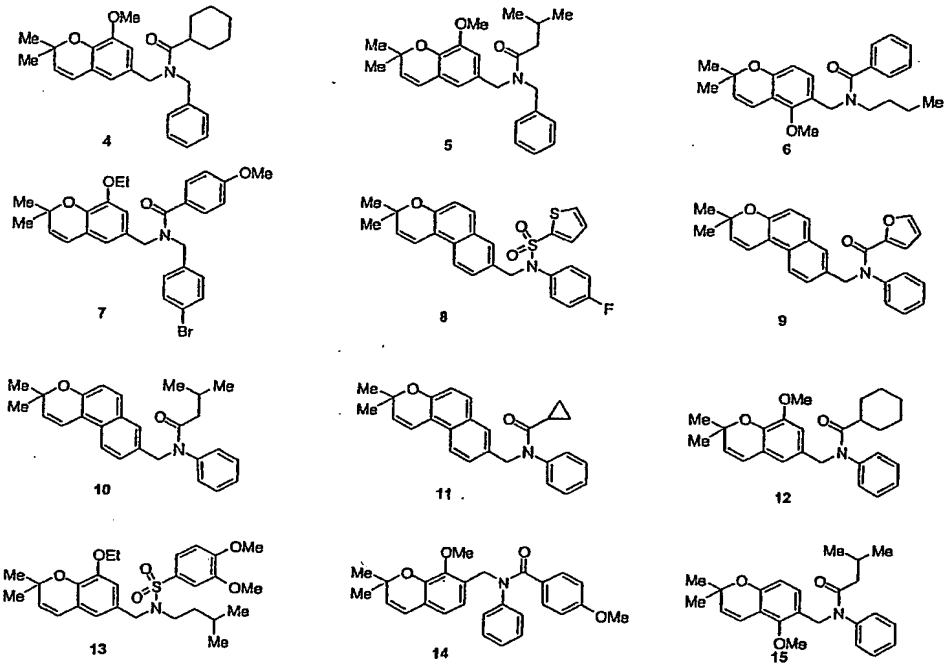


Figure 3(a)

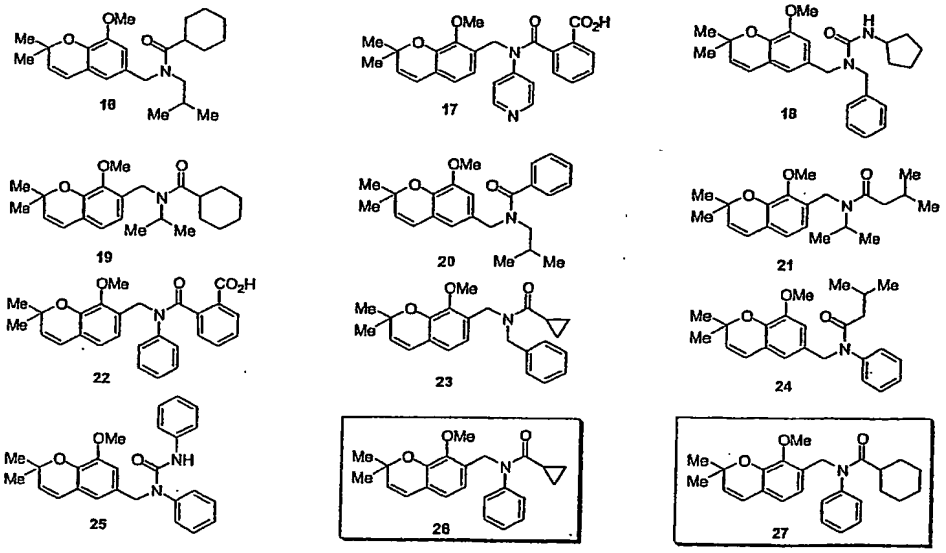


Figure 3(b)

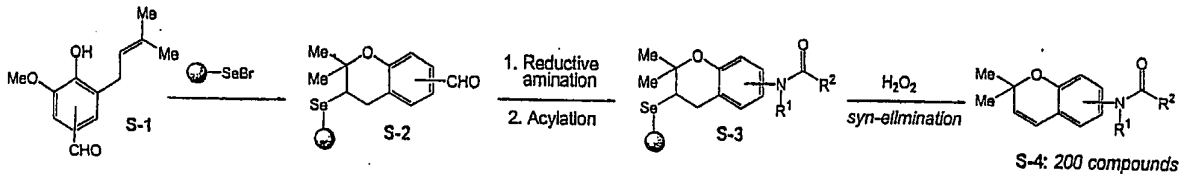


Figure 4(a)

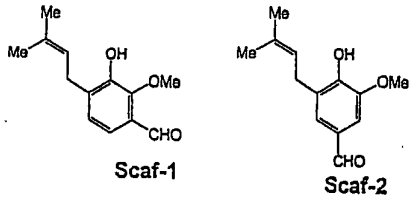


Figure 4(b)

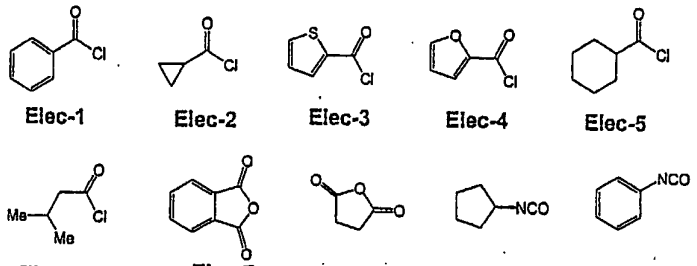


Figure 4(c)

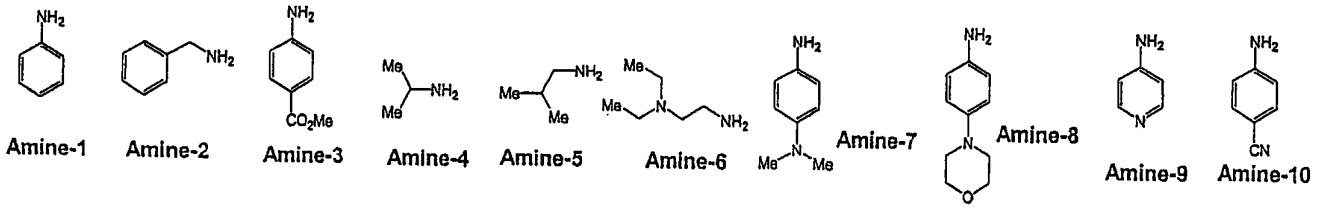


Figure 4(d)

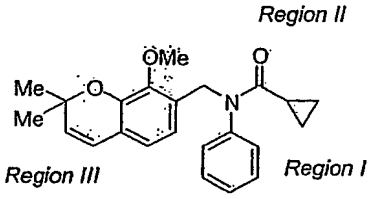


Figure 5

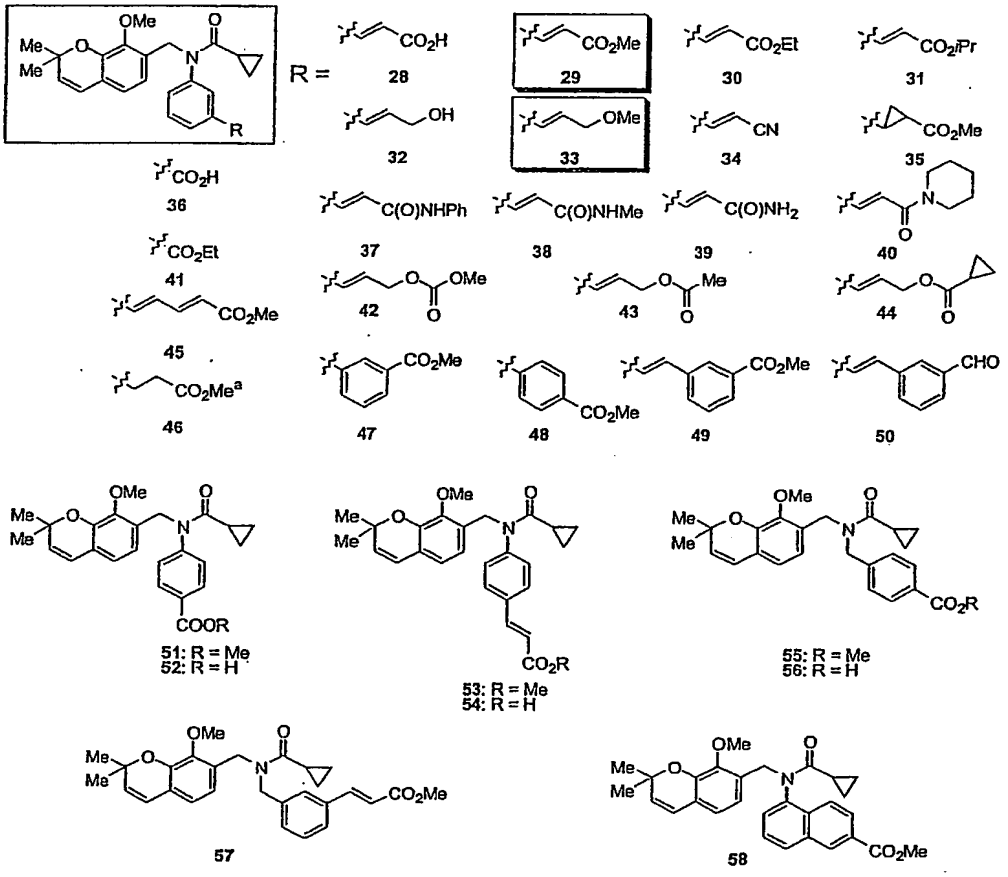


Figure 6

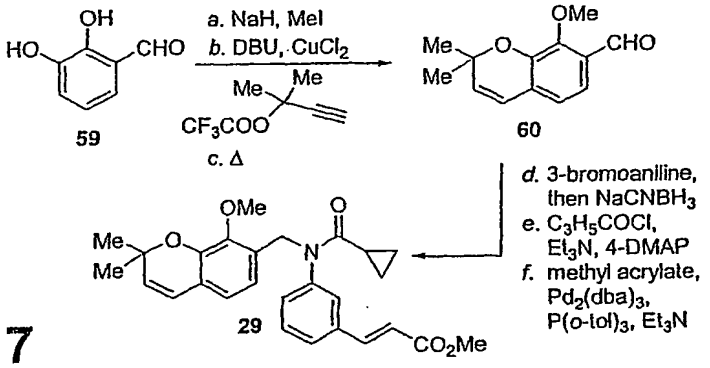


Figure 7

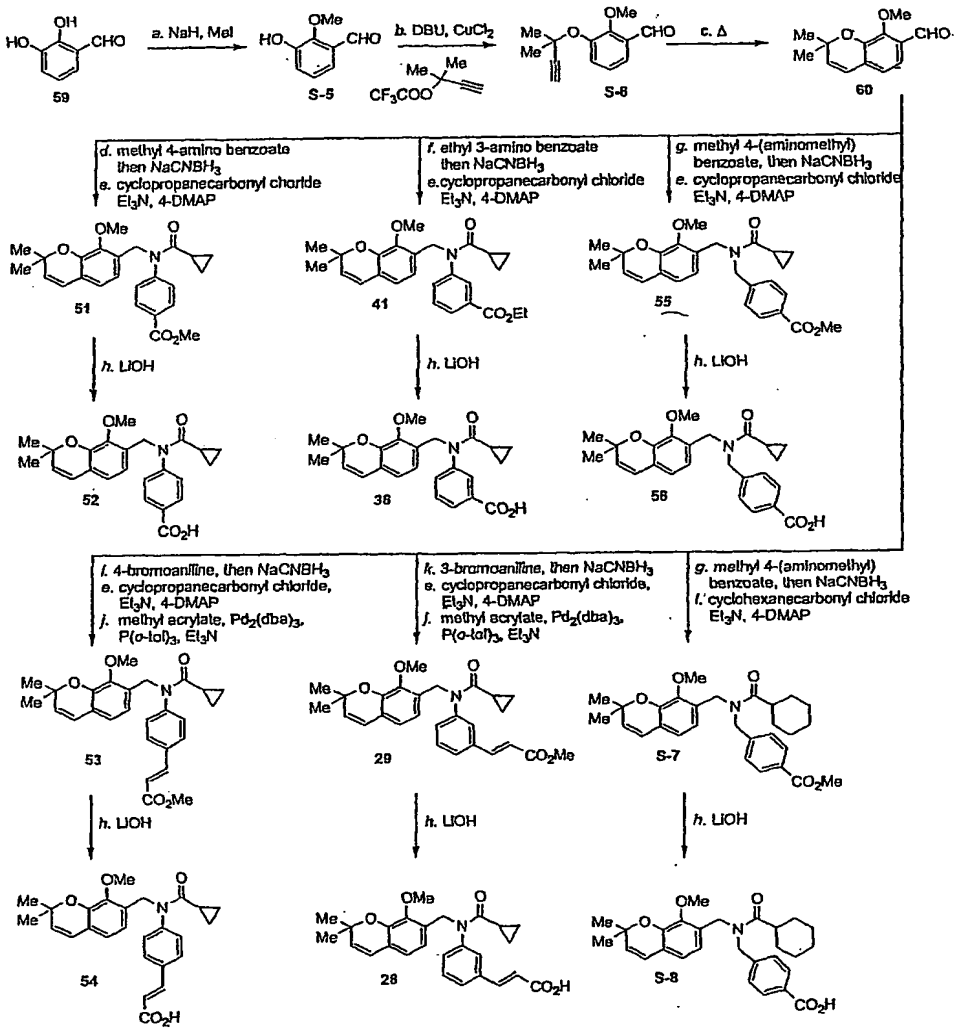


Figure 8

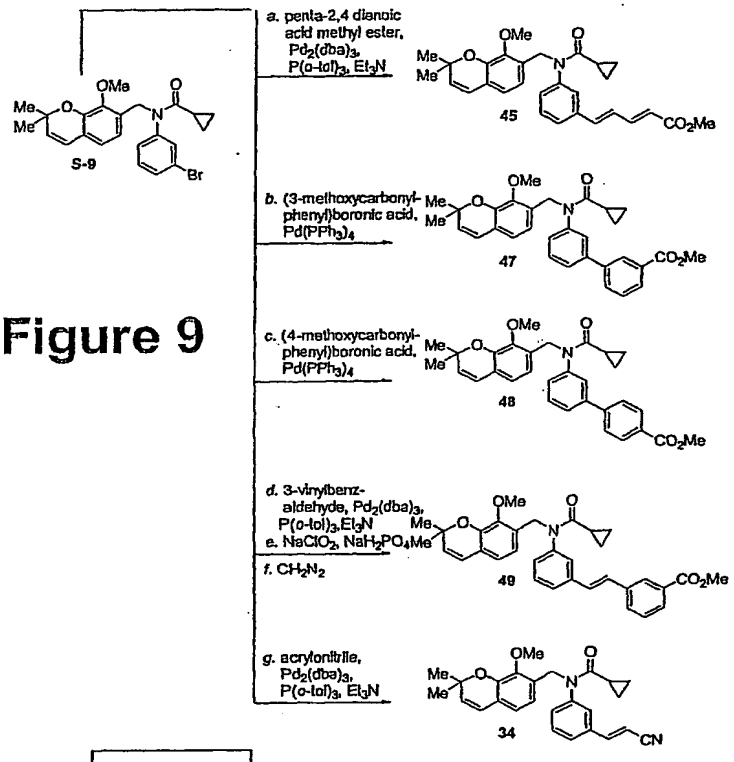


Figure 9

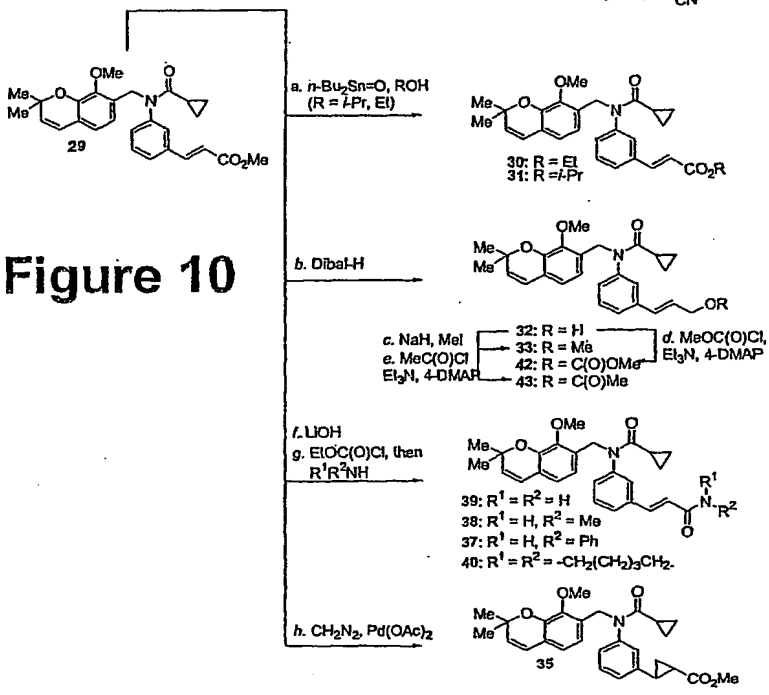


Figure 10

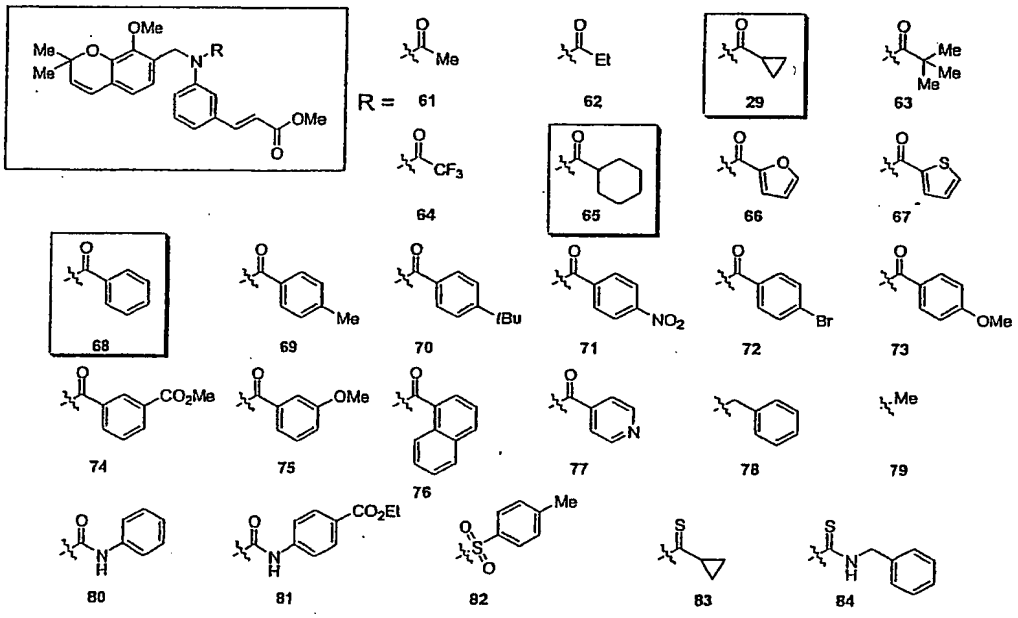

R =
61
62
29
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84

Figure 11

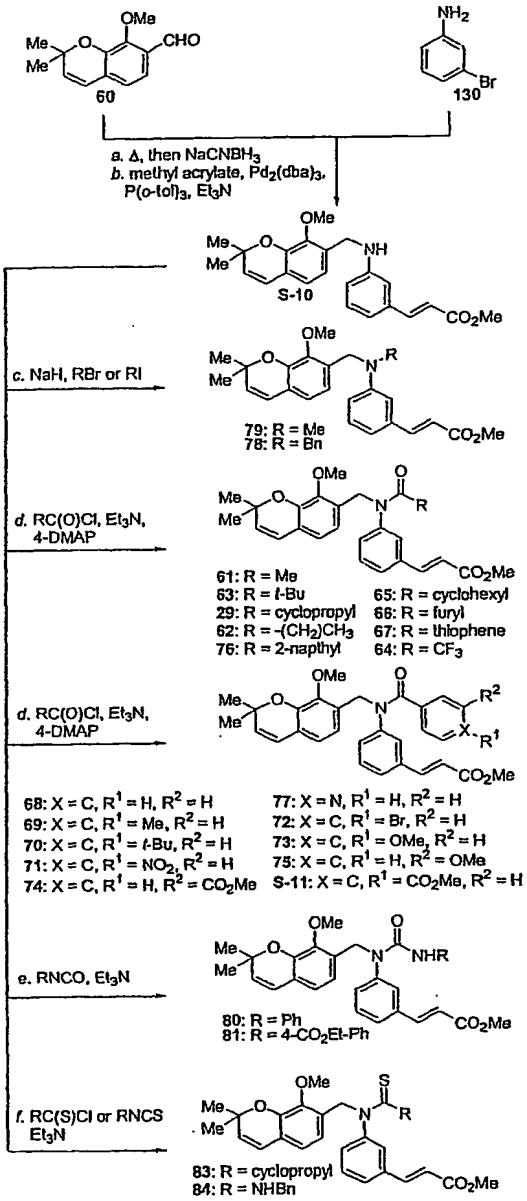

OMe
Me
Me
O
CHO
60
NH2
Br
130
a. Δ, then NaCNBH3
b. methyl acrylate, Pd2(dba)3, P(o-tol)3, Et3N
NH
S-10
CO2Me
c. NaH, RBr or RI
N-R
79: R = Me
78: R = Bn
d. RC(O)Cl, Et3N, 4-DMAP
61: R = Me
63: R = t-Bu
29: R = cyclopropyl
62: R = -(CH2)CH3
76: R = 2-napthyl
65: R = cyclohexyl
66: R = furyl
67: R = thiophene
64: R = CF3
68: X = C, R1 = H, R2 = H
69: X = C, R1 = Me, R2 = H
70: X = C, R1 = t-Bu, R2 = H
71: X = C, R1 = NO2, R2 = H
74: X = C, R1 = H, R2 = CO2Me
77: X = N, R1 = H, R2 = H
72: X = C, R1 = Br, R2 = H
73: X = C, R1 = OMe, R2 = H
75: X = C, R1 = H, R2 = OMe
S-11: X = C, R1 = CO2Me, R2 = H
e. RNCO, Et3N
NHR
80: R = Ph
81: R = 4-CO2Et-Ph
f. RC(S)Cl or RNCS Et3N
S
83: R = cyclopropyl
84: R = NHBn

Figure 12

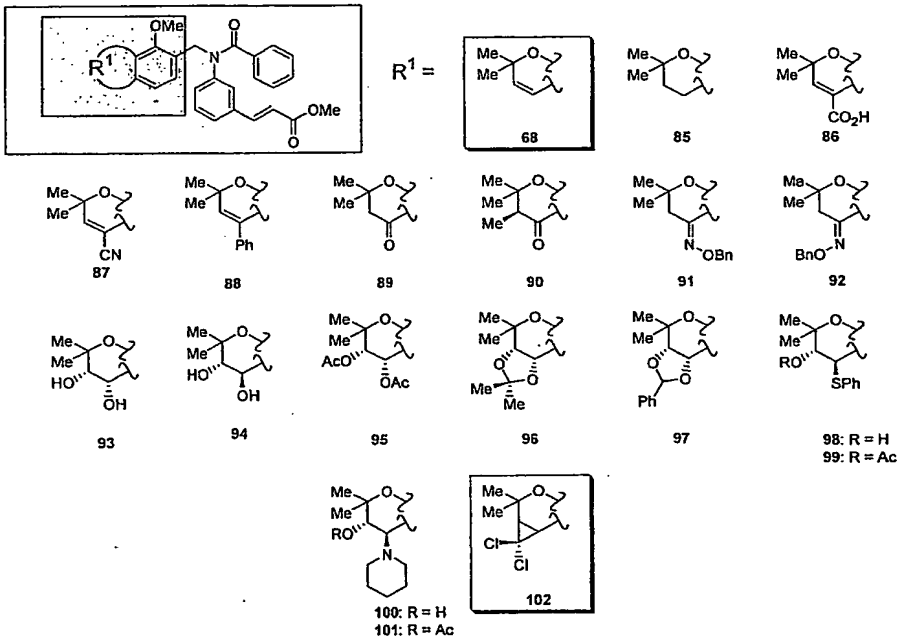

R1 =
68
85
86
87
88
89
90
91
92
93
94
95
96
97
98: R = H
99: R = Ac
100: R = H
101: R = Ac
102

Figure 13

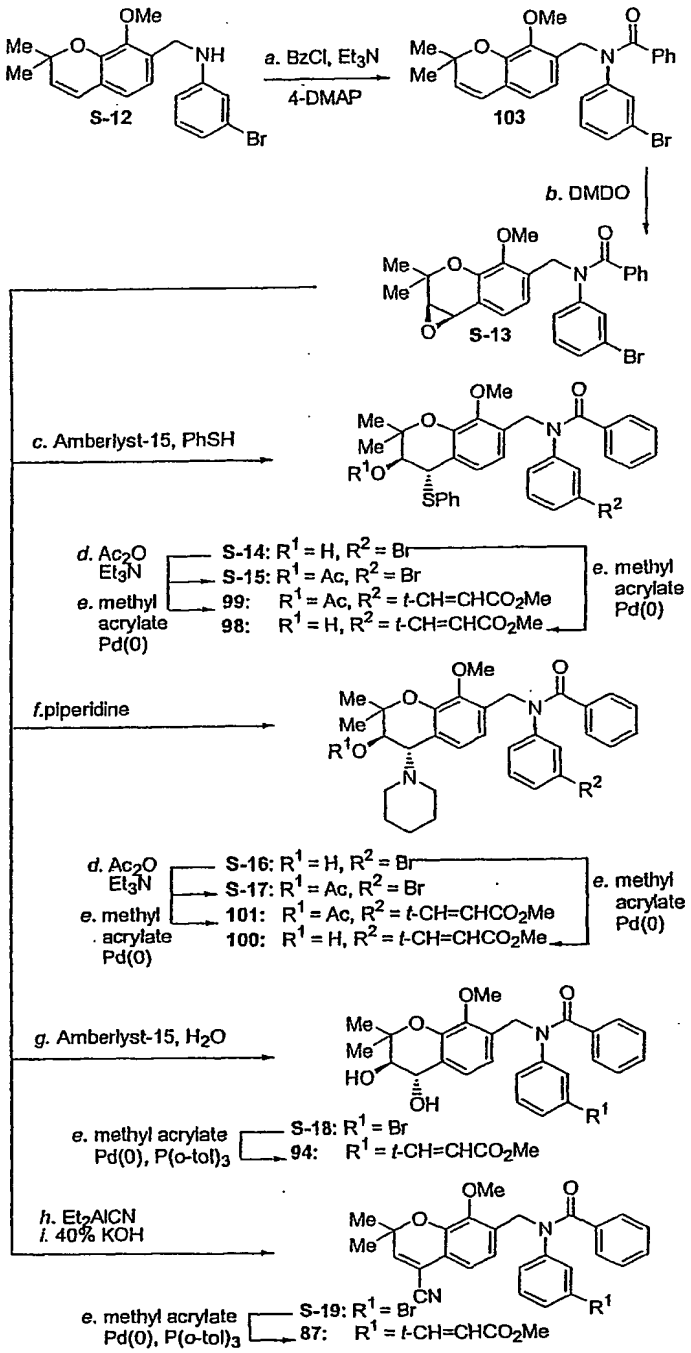

OMe
Me
Me
O
NH
Br
S-12
a. BzCl, Et3N
4-DMAP
103
Ph
b. DMDO
S-13
c. Amberlyst-15, PhSH
SPh
R1O
R2
d. Ac2O
Et3N
e. methyl
acrylate
Pd(0)
S-14: R1 = H, R2 = Br
S-15: R1 = Ac, R2 = Br
99: R1 = Ac, R2 = t-CH=CHCO2Me
98: R1 = H, R2 = t-CH=CHCO2Me
f.piperidine
S-16: R1 = H, R2 = Br
S-17: R1 = Ac, R2 = Br
101: R1 = Ac, R2 = t-CH=CHCO2Me
100: R1 = H, R2 = t-CH=CHCO2Me
g. Amberlyst-15, H2O
HO
OH
R1
S-18: R1 = Br
94: R1 = t-CH=CHCO2Me
h. Et2AlCN
i. 40% KOH
CN
S-19: R1 = Br
87: R1 = t-CH=CHCO2Me

Figure 14

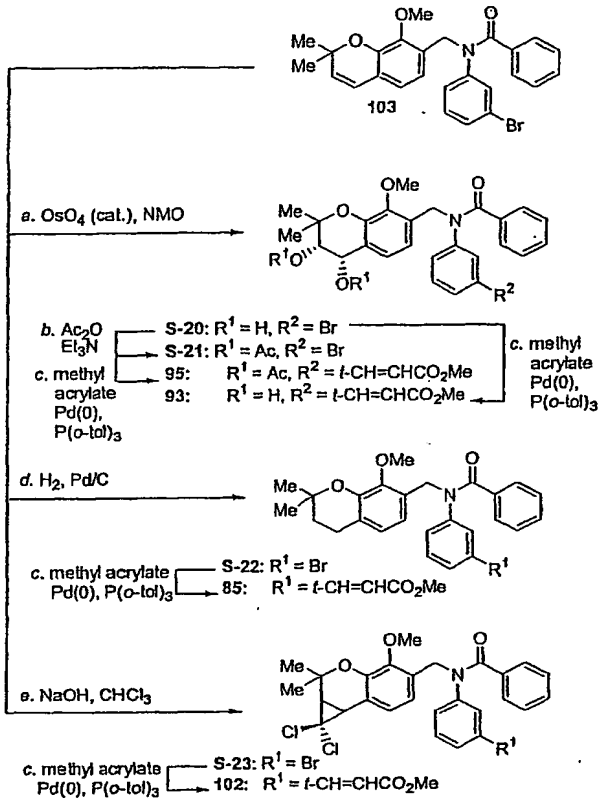


Figure 15

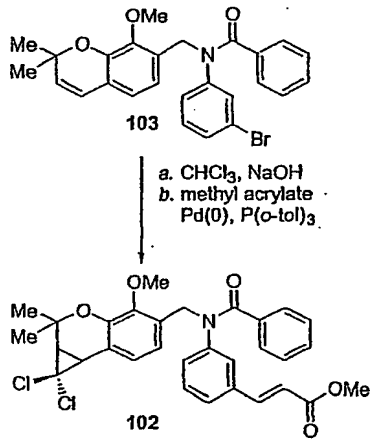


Figure 16

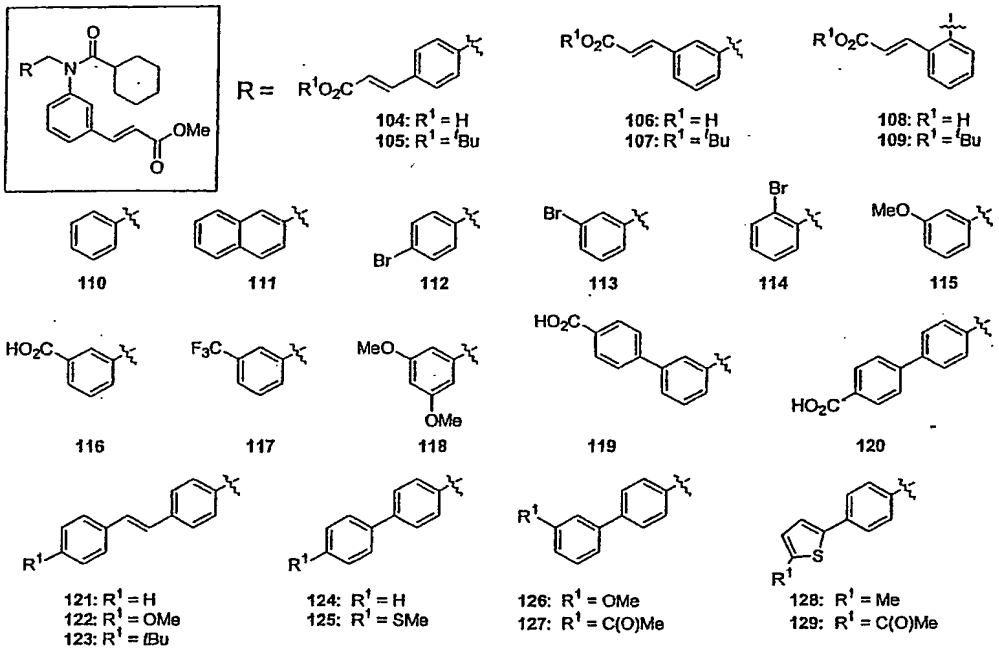


Figure 17

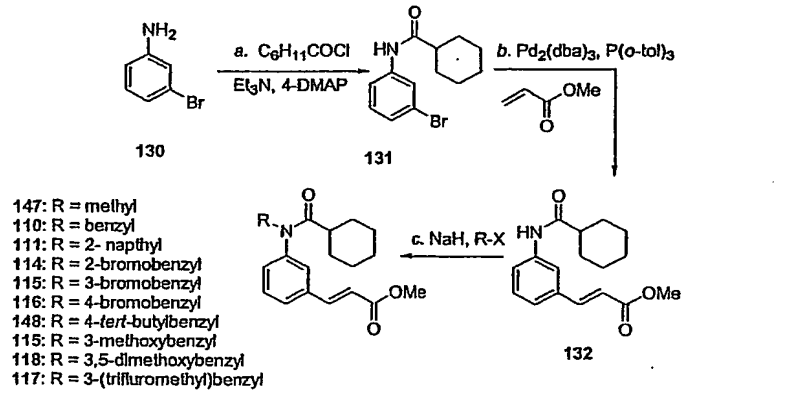


Figure 18

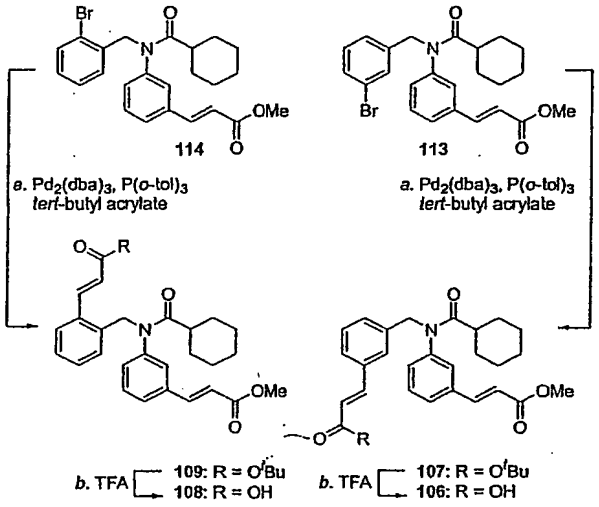
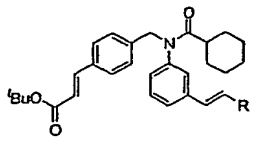
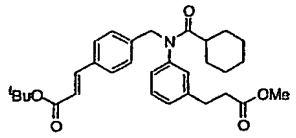

Br
O
N
OMe
O
114
113
a. $Pd_2(dba)_3$, P(o-tol)$_3$
tert-butyl acrylate
R
b. TFA
109: R = O^{t}Bu
108: R = OH
107: R = O^{t}Bu
106: R = OH

Figure 19

a. SAR of region I



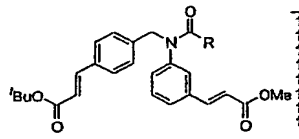
	R	EC_{50} (nM)	RE[a]
105	COOMe	127	2.12
133	COOEt	256	2.07
134	COO^{t}Bu	>1000	1.06
135	$CONH_2$	>1000	0.50
136	CH_2OMe	243	1.68
137	CH_2OEt	220	1.74
138	CH_2OPh	2830	0.45



139: EC_{50} = 274 nM
RE[a] = 1.38

Figure 20(a)

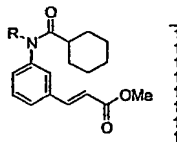
b. SAR of region II



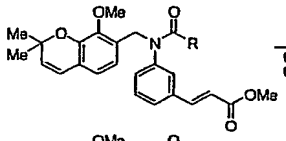
	R	EC_{50} (nM)	RE[a]
140	cyclopropyl	250	1.68
141	cyclobutyl	187	1.84
142	cyclopentyl	162	2.16
105	cyclohexyl	127	2.12
143	phenyl	238	1.96
144	2-furyl	205	1.93
145	isopropylamino	212	1.96
146	benzylamino	>1000	0.27

Figure 20(b)

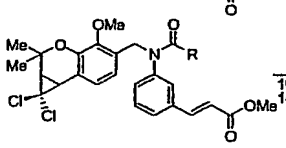
c. SAR of region III



	R	EC_{50} (nM)	RE[a]
132	H	>1000	0.09
147	methyl	>1000	0.09
110	benzyl	>1000	0.09
111	2- napthyl	680	0.41
114	2-bromobenzyl	>1000	0.11
113	3-bromobenzyl	>1000	0.10
112	4-bromobenzyl	>1000	0.28
148	4-*tert*-butylbenzyl	>1000	0.15
115	3-methoxybenzyl	>1000	0.11
118	3,5-dimethoxybenzyl	608	0.12
117	3-(trifluromethyl)benzyl	>1000	



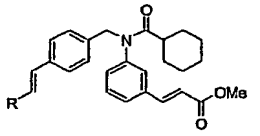
	R	EC_{50} (nM)	RE[a]
68	phenyl	>1000	0.83
65	cyclohexyl	358	0.40



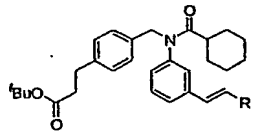
	R	EC_{50} (nM)	RE[a]
102	phenyl	333	0.64
149	cyclohexyl	188	0.50

Figure 20(c)

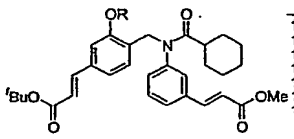
d. SAR of region III



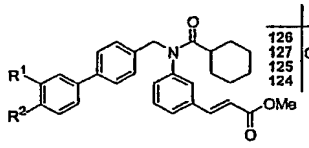
	R	EC_{50} (nM)	RE[a]
104	COOH	>1000	0.08
150	COOMe	>1000	0.87
151	COOEt	>1000	1.14
152	COO^{i}Pr	163	1.97
105	COO^{t}Bu	127	2.12
153	COOBn	>1000	0.23
154	$CONMe_2$	>1000	0.66
155	CONH^{t}Bu	>1000	1.65
156	CH_2OMe	233	1.63
157	CH_2OEt	198	2.06
158	CH_2OPh	>1000	0.64



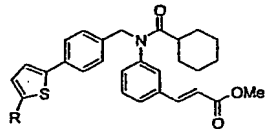
	R	EC_{50} (nM)	RE[a]
159	COOMe	240	1.56
160	COO^{t}Bu	>1000	0.64



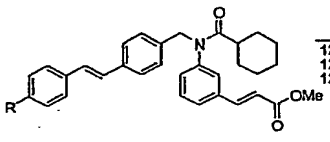
	R	EC_{50} (nM)	RE[a]
161	H	> 1000	0.12
162	Me	> 1000	0.14
163	Bn	> 1000	0.38
164	MeC(O)	> 1000	0.16
165	$C_6H_5C(O)$	> 1000	0.18
166	$MeS(O_2)$	> 1000	0.18
167	$EtOOCCH_2$	> 1000	



	R^1	R^2	EC_{50} (nM)	RE[a]
126	OMe	H	77	1.51
127	C(O)Me	H	227	1.30
125	H	SMe	69	1.74
124	H	H	510	0.71



	R	EC_{50} (nM)	RE[a]
128	Me	206	1.78
129	C(O)Me	256	1.48



	R	EC_{50} (nM)	RE[a]
121	H	38	1.55
122	OMe	208	1.67
123	t-Bu	>1000	0.29

Figure 20(d)

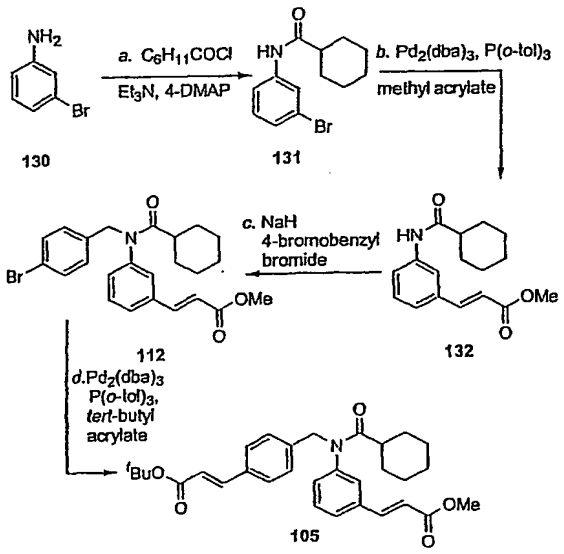


Figure 21

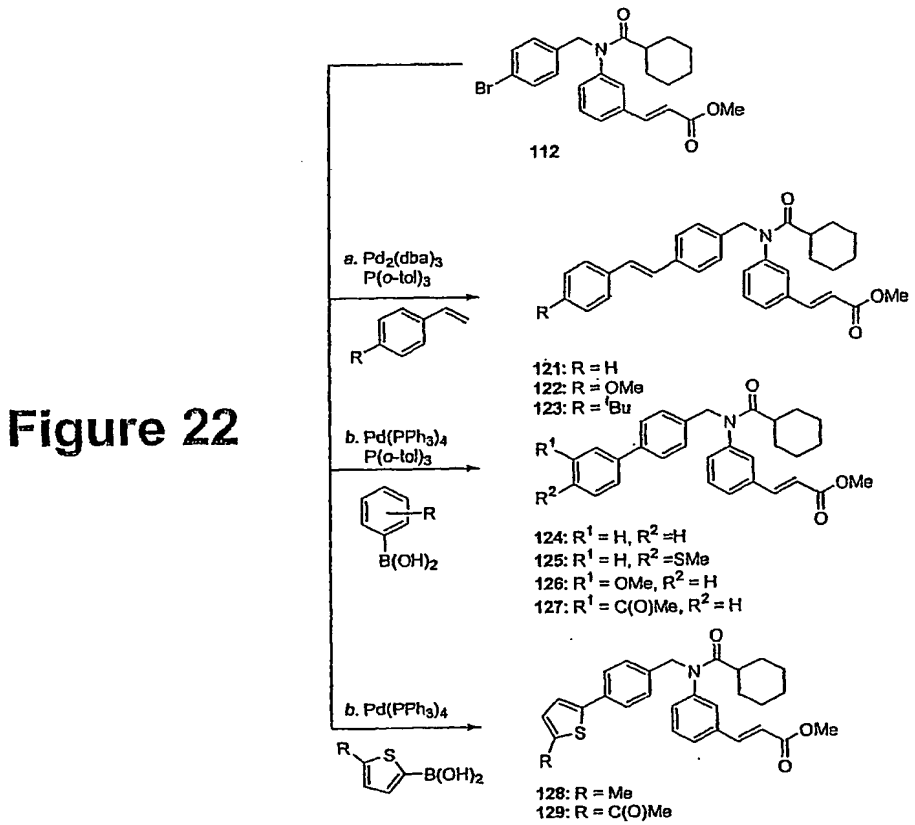


Figure 22

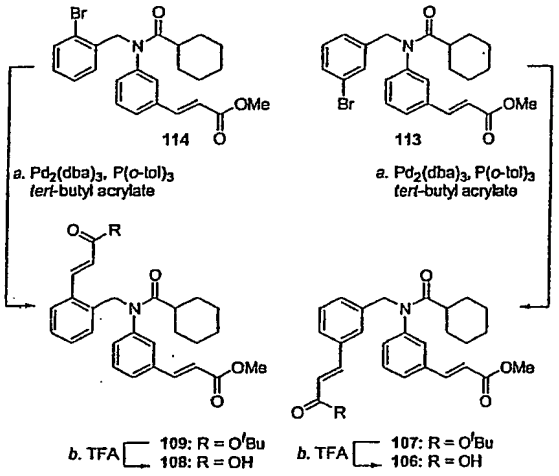


Figure 23

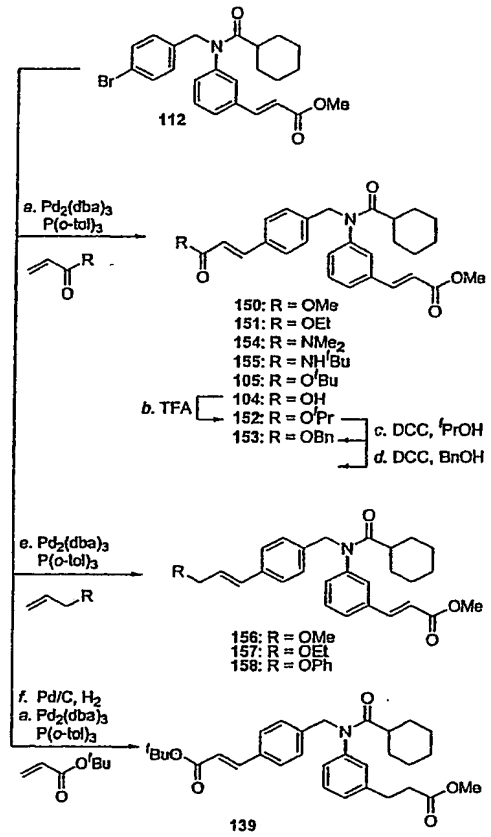


Figure 24

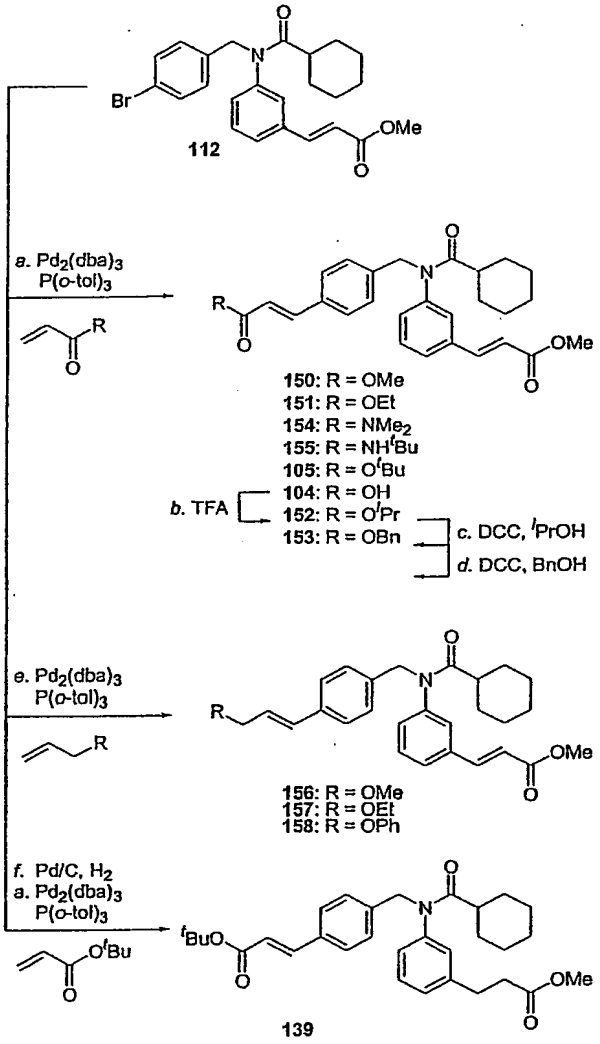

Br
112
OMe
a. Pd2(dba)3
P(o-tol)3
150: R = OMe
151: R = OEt
154: R = NMe2
155: R = NHtBu
105: R = OtBu
b. TFA
104: R = OH
152: R = OiPr
153: R = OBn
c. DCC, iPrOH
d. DCC, BnOH
e. Pd2(dba)3
P(o-tol)3
156: R = OMe
157: R = OEt
158: R = OPh
f. Pd/C, H2
a. Pd2(dba)3
P(o-tol)3
tBuO
OtBu
139

Figure 25

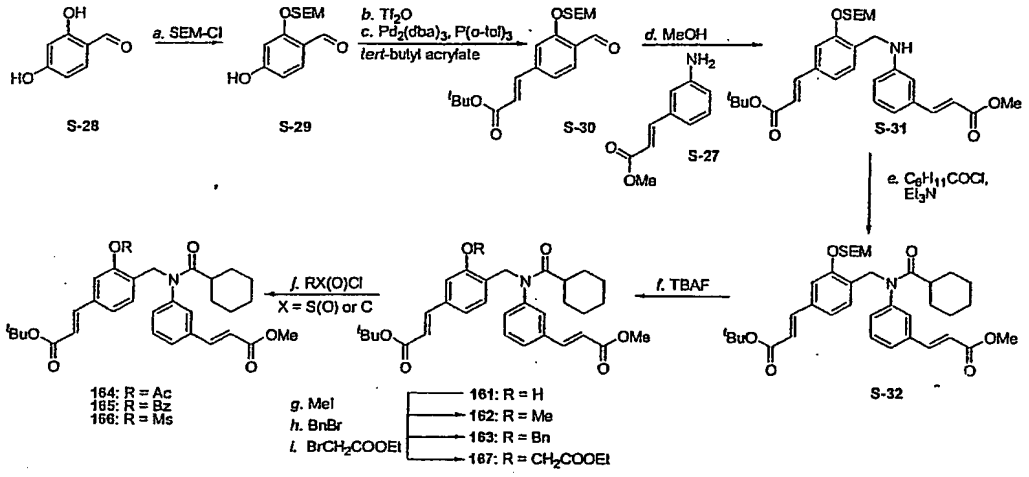


Figure 26

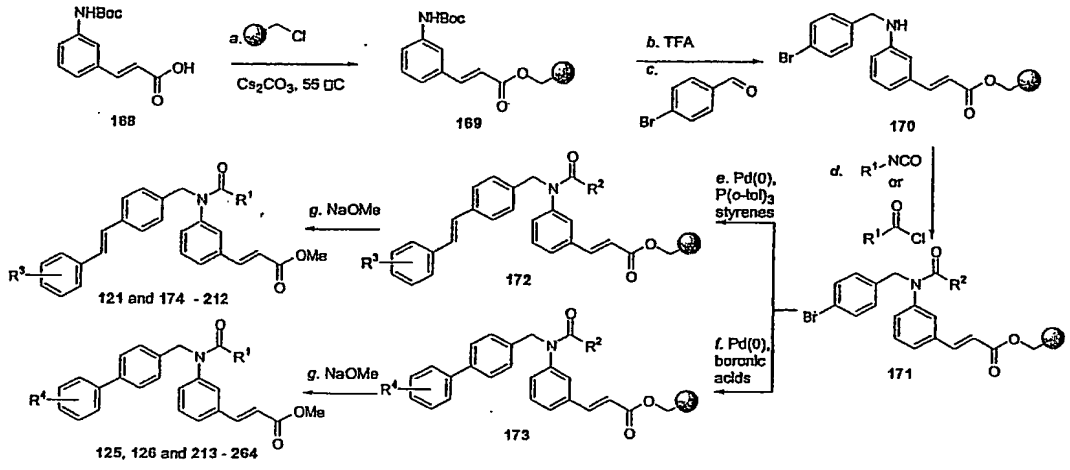
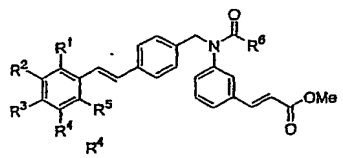
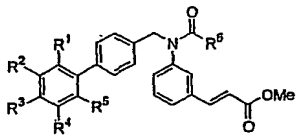
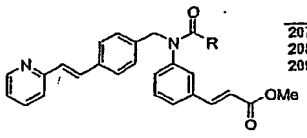


Figure 27

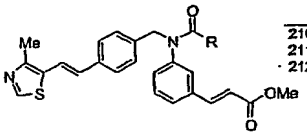




	R^1	R^2	R^3	R^4	R^5	R^6	EC_{50} (nM)	RE[a]
174	H	H	Me	H	H	$-C_6H_{11}$	342	0.83
175	H	H	Me	H	H	$-CH(CH_3)_2$	1410	0.37
176	H	H	Me	H	H	$-NHCH(CH_3)_2$	3570	0.10
177	Cl	H	H	H	Cl	$-C_6H_{11}$	150	0.12
178	Cl	H	H	H	Cl	$-CH(CH_3)_2$	195	0.14
179	Cl	H	H	H	Cl	$-NHCH(CH_3)_2$	216	0.15
180	H	Cl	H	H	H	$-C_6H_{11}$	165	1.41
181	H	Cl	H	H	H	$-CH(CH_3)_2$	164	1.09
182	H	Cl	H	H	H	$-NHCH(CH_3)_2$	339	0.59
183	H	CF_3	H	CF_3	H	$-C_6H_{11}$	1470	0.15
184	H	CF_3	H	CF_3	H	$-CH(CH_3)_2$	1950	0.13
185	H	CF_3	H	CF_3	H	$-NHCH(CH_3)_2$	1830	0.13
186	H	CF_3	H	H	H	$-C_6H_{11}$	937	0.35
187	H	CF_3	H	H	H	$-CH(CH_3)_2$	267	0.70
188	H	CF_3	H	H	H	$-NHCH(CH_3)_2$	932	0.31
189	F	H	H	H	F	$-C_6H_{11}$	174	0.94
190	F	H	H	H	F	$-CH(CH_3)_2$	108	0.79
191	F	H	H	H	F	$-NHCH(CH_3)_2$	4020	0.21
192	F	H	H	H	H	$-C_6H_{11}$	64	1.41
193	F	H	H	H	H	$-CH(CH_3)_2$	70	1.17
194	F	H	H	H	H	$-NHCH(CH_3)_2$	431	0.69
195	Me	H	Me	H	Me	$-C_6H_{11}$	518	0.24
196	Me	H	Me	H	Me	$-CH(CH_3)_2$	149	0.30
197	Me	H	Me	H	Me	$-NHCH(CH_3)_2$	431	0.14
121	H	H	H	H	H	$-C_6H_{11}$	36	1.55
198	H	H	H	H	H	$-CH(CH_3)_2$	65	1.33
200	H	H	H	H	H	$-NHCH(CH_3)_2$	119	1.38
201	H	F	H	H	H	$-C_6H_{11}$	66	1.36
202	H	F	H	H	H	$-CH(CH_3)_2$	71	1.33
203	H	F	H	H	H	$-NHCH(CH_3)_2$	467	0.61
204	H	H	F	H	H	$-C_6H_{11}$	185	0.53
205	H	H	F	H	H	$-CH(CH_3)_2$	120	1.19
206	H	H	F	H	H	$-NHCH(CH_3)_2$	348	0.91



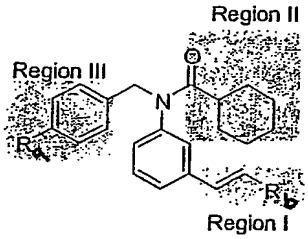
	R	EC_{50} (nM)	RE[a]
207	$-C_6H_{11}$	309	0.81
208	$-CH(CH_3)_2$	310	0.62
209	$-NHCH(CH_3)_2$	575	0.66



	R	EC_{50} (nM)	RE[a]
210	$-C_6H_{11}$	227	0.53
211	$-CH(CH_3)_2$	228	0.32
212	$-NHCH(CH_3)_2$	366	0.42

	R^1	R^2	R^3	R^4	R^5	R^6	EC_{50} (nM)	RE[a]
213	H	F	F	H	H	$-C_6H_{11}$	72	1.70
214	H	F	F	H	H	$-CH(CH_3)_2$	249	1.15
215	H	F	F	H	H	$-NHCH(CH_3)_2$	8180	0.23
125	H	H	SMe	H	H	$-C_6H_{11}$	69	1.74
216	H	H	SMe	H	H	$-CH(CH_3)_2$	51	0.98
217	H	H	SMe	H	H	$-NHCH(CH_3)_2$	178	0.23
218	OMe	H	H	H	H	$-C_6H_{11}$	359	0.49
219	OMe	H	H	H	H	$-CH(CH_3)_2$	377	0.28
220	OMe	H	H	H	H	$-NHCH(CH_3)_2$	4010	0.09
126	H	Cl	H	Cl	H	$-C_6H_{11}$	284	0.95
221	H	Cl	H	Cl	H	$-CH(CH_3)_2$	661	0.54
222	H	Cl	H	Cl	H	$-NHCH(CH_3)_2$	>10000	0.10
223	H	OMe	H	H	H	$-C_6H_{11}$	101	1.51
224	H	OMe	H	H	H	$-CH(CH_3)_2$	72	1.26
225	H	OMe	H	H	H	$-NHCH(CH_3)_2$	1370	0.41
226	H	OEt	H	H	H	$-C_6H_{11}$	147	1.37
227	H	OEt	H	H	H	$-CH(CH_3)_2$	173	1.03
228	H	OEt	H	H	H	$-NHCH(CH_3)_2$	2350	0.33
229	H	H	OMe	H	H	$-C_6H_{11}$	89	1.71
230	H	H	OMe	H	H	$-CH(CH_3)_2$	97	1.21
231	H	H	OMe	H	H	$-NHCH(CH_3)_2$	144	1.16
232	H	Cl	H	H	H	$-C_6H_{11}$	94	1.56
233	H	Cl	H	H	H	$-CH(CH_3)_2$	77	1.52
234	H	Cl	H	H	H	$-NHCH(CH_3)_2$	1400	0.49
235	H	H	Me	H	H	$-C_6H_{11}$	26	1.38
236	H	H	Me	H	H	$-CH(CH_3)_2$	118	1.48
237	H	H	Me	H	H	$-NHCH(CH_3)_2$	449	0.80
238	H	Me	H	H	H	$-C_6H_{11}$	109	1.43
239	H	Me	H	H	H	$-CH(CH_3)_2$	163	1.09
240	H	Me	H	H	H	$-NHCH(CH_3)_2$	1330	0.53
241	OMe	H	H	Cl	H	$-C_6H_{11}$	233	1.16
242	OMe	H	H	Cl	H	$-CH(CH_3)_2$	226	0.79
243	OMe	H	H	Cl	H	$-NHCH(CH_3)_2$	3080	0.17
244	H	$-OCH_2O-$		H	H	$-C_6H_{11}$	38	1.90
245	H	$-OCH_2O-$		H	H	$-CH(CH_3)_2$	19	1.25
246	H	$-OCH_2O-$		H	H	$-NHCH(CH_3)_2$	96	1.51
247	H	Cl	F	H	H	$-C_6H_{11}$	66	1.87
248	H	Cl	F	H	H	$-CH(CH_3)_2$	129	1.64
249	H	Cl	F	H	H	$-NHCH(CH_3)_2$	3050	0.41
250	H	H	OCF_3	H	H	$-C_6H_{11}$	264	1.04
251	H	H	OCF_3	H	H	$-CH(CH_3)_2$	219	0.78
252	H	H	OCF_3	H	H	$-NHCH(CH_3)_2$	7530	0.21
253	H	OCF_3	H	H	H	$-C_6H_{11}$	420	0.84
254	H	OCF_3	H	H	H	$-CH(CH_3)_2$	247	0.69
255	H	OCF_3	H	H	H	$-NHCH(CH_3)_2$	>10000	0.09
256	OMe	H	H	H	OMe	$-C_6H_{11}$	77	0.12
257	OMe	H	H	H	OMe	$-CH(CH_3)_2$	95	0.10
258	OMe	H	H	H	OMe	$-NHCH(CH_3)_2$	581	0.10
259	H	H	NMe_2	H	H	$-C_6H_{11}$	25	1.72
260	H	H	NMe_2	H	H	$-CH(CH_3)_2$	57	1.07
261	H	H	NMe_2	H	H	$-NHCH(CH_3)_2$	162	1.01
262	H	H	*t*-Bu	H	H	$-C_6H_{11}$	132	1.38
263	H	H	*t*-Bu	H	H	$-CH(CH_3)_2$	343	0.59
264	H	H	*t*-Bu	H	H	$-NHCH(CH_3)_2$	262	1.02

Figure 28



Region I: Methyl acrylate or allylic methyl ether necessary for optimum activity. In some instances, when other areas were optimized, olefin can be removed while retaining some potency.
Region II: Amide or urea essential for maximum activity. Alkyl or cycloalkyl amide or urea affords most potent compounds.
Region III: Must have para-position functionalized for activity. Steric bulk and length seem to be the most important factors whcih govern potency. This region is tolerant of many different structural motifs.

Figure 29

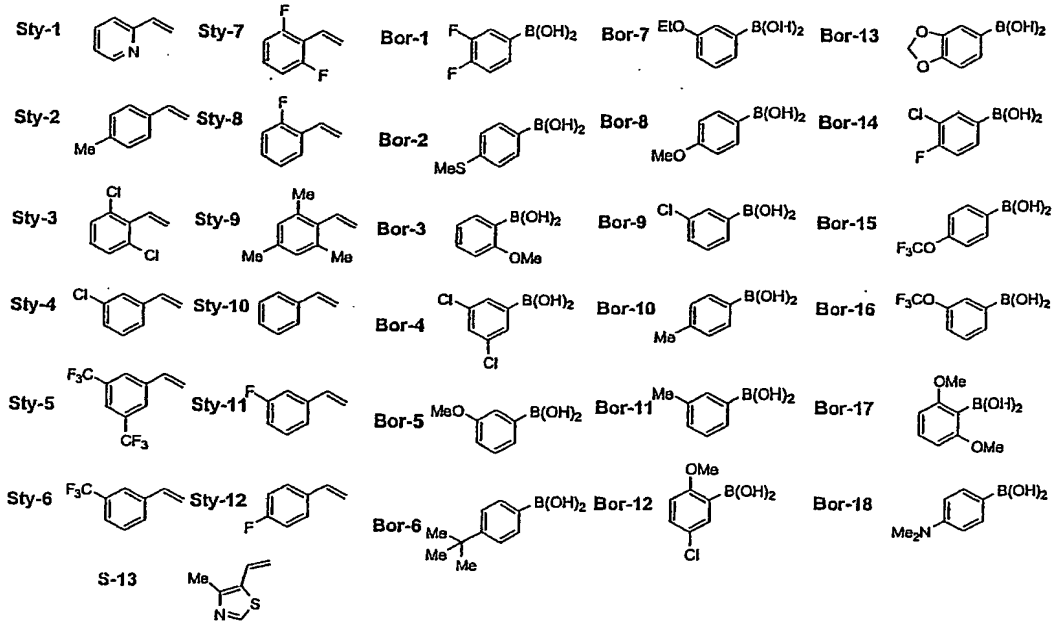


Figure 30

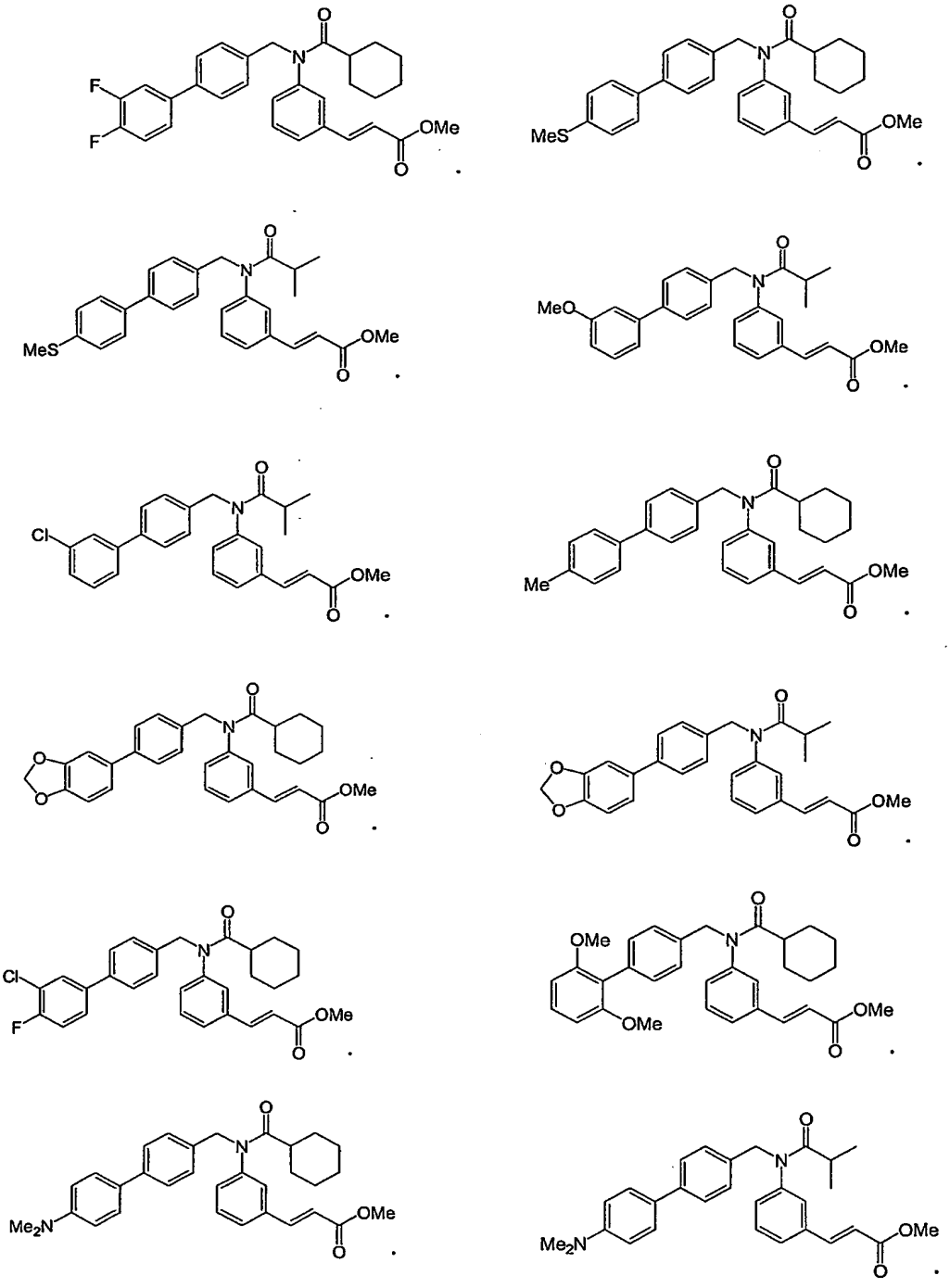

F
F
OMe
MeS
OMe
MeS
OMe
MeO
OMe
Cl
OMe
Me
OMe
OMe
OMe
Cl
F
OMe
OMe
OMe
Me2N
OMe
Me2N
OMe

Figure 31

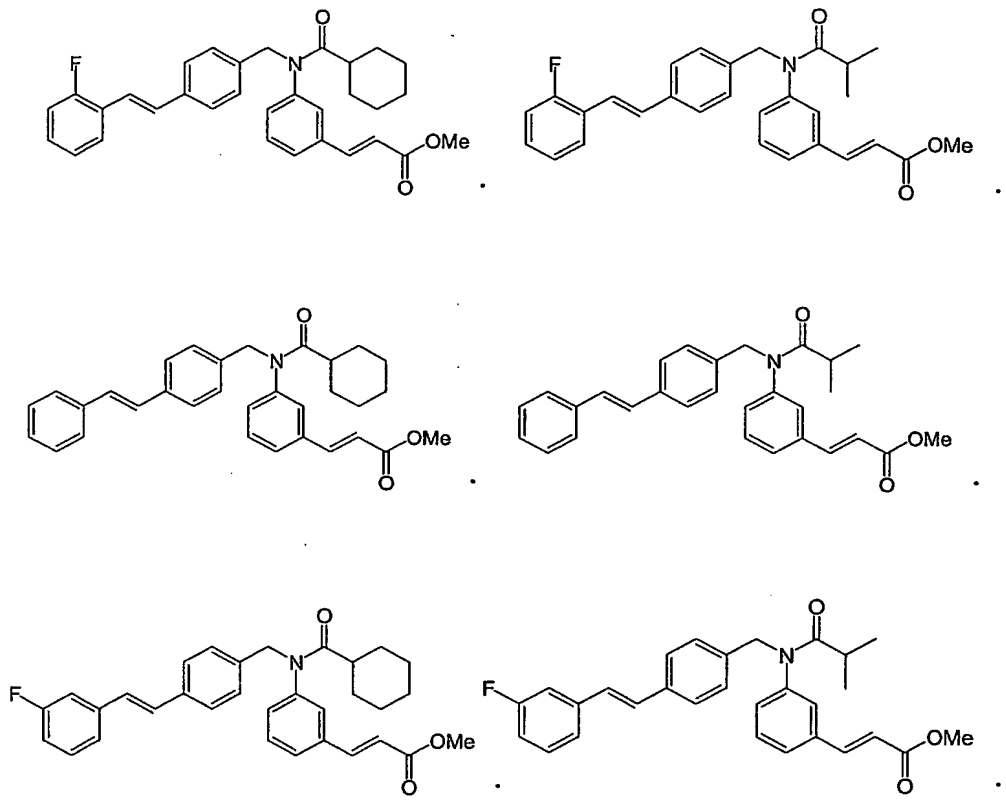
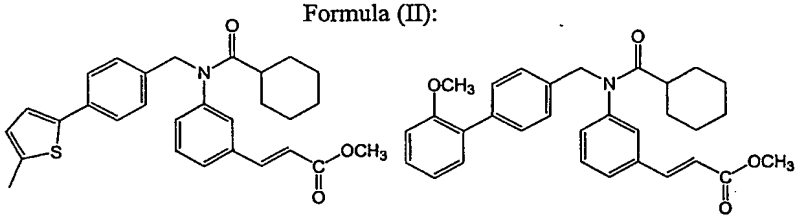
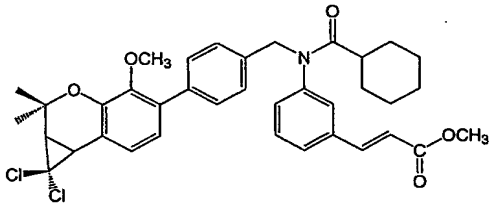


Figure 32

Formula (II):



Formula (IV):



Formula (V):

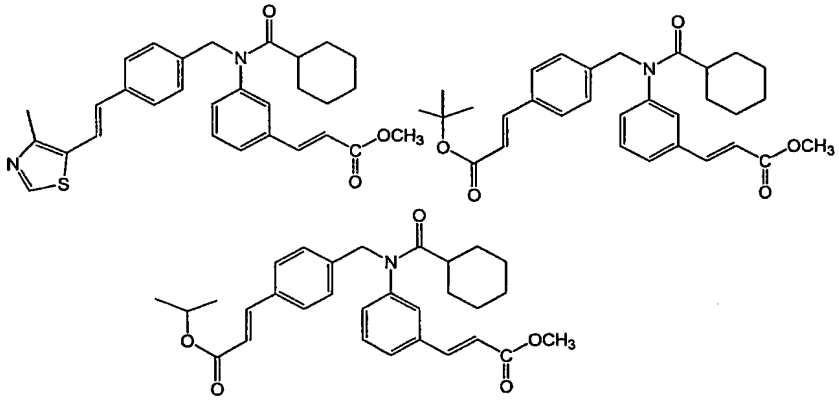


Figure 33

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/36195

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C07C 233/61
US CL : 564/123
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 564/123

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database CAS ONLINE on STN, Chem. Abstr., accession No. 1995:559328, BECKWITH et al. "Tandem radical translocation and homolytic aromatic substitution: a convenient and efficient route to oxindoles". Journal of the Chemical Society, Chemical Communications (1995), Vol. 9, pages 977-8, abstract.	5 and 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
18 March 2004 (18.03.2004)

Date of mailing of the international search report
21 MAY 2004

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/36195

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claim Nos.: 1-4 and 7-32
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Please See Continuation Sheet

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US03/36195

Continuation of Box I Reason 2:
The numerous variables, e.g. A, E1, L1, L2, X, Y, Z1-Z10, G1-G18, T, A2, etc., and their voluminous, complex meanings and their virtual incomprehensible permutations and combinations make it impossible to determine the full scope and complete meaning of the claimed subject matter. As presented the claimed subject matter cannot be regarded as being a clear and concise description for which protection is sought and as such the listed claims do not comply with the requirements of PCT Article 6. Thus it is impossible to carry out a meaningful search on same. A search will be performed on the first discernable invention which is the invention of claims 5 and 6.

Continuation of B. FIELDS SEARCHED Item 3:
CAS ONLINE
STN structure search